7th BRATISLAVA SYMPOSIUM ON SACCHARIDES

PROGRAMME AND ABSTRACTS

Smolenice, SLOVAKIA

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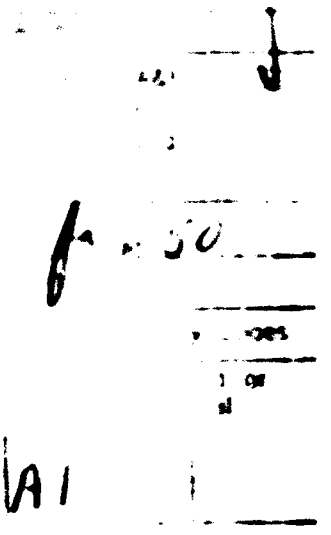

August 29-September 2, 1994
Smolenice, Slovakia

7th BRATISLAVA SYMPOSIUM ON SACCHARIDES

is organised by the Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia, at the occasion of the 40th anniversary of the Institute's foundation

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2. Gajdoš J., Ferro D.R., Ragazzi M.:
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3. Hricovíni M., Guerrini M., Torri G., Piani S., Ungarelli F.:
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4. Kačuráková M., Mathlouthi M., Hirsch J., Ebringerová A.:
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5. Mathlouthi M., Kačuráková M.:
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6. Malovíková A., Rinaudo M., Milas M.:
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7. Naran R., Ebringerová A., Alföldi J., Pätoprsty V.:
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9. Hromádková Z., Ebringerová A., Malovíková A., Burchard W.:
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12. Košíková B., Hricovíni M., Simonutti R.:
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13. Joniak D., Košíková B.:
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14. Panina N.I., Dubina L.G., Khomutov L.I.:
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15. Nahálka J., Welwardová A., Gemeiner P., Nahálková I., Rosenberg M., Šturdík E.:
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19. Hagarová D., Breier A., Gemeiner P.:
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21. Pátoprstý V., Pribulová B., Königstein J.:
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4 Bronšlová A., Šandula J., Slováková L.
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5 Farkašová Z., Šoltés L., Machová E.
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8. Škultéty Ľ., Toman R.
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9. Duskhin M.I., Safina A.F., Vereschagin E.I., Korolenko T.A.
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10. Zabotin A., Barisheva T., Zabotina O., Larskaya I., Pivovarov M., Beldman G., Lozovaya V.:
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11. Zabotina O.A., Gurjanov O.P., Ayupova D.A., Larskaya I.A., Beldman G., Lozovaya V.V.:
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12. Zabotina O.A., Malyhov R.G., Schols H.A., Beldman G., Lozovaya V.V.:
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13. Auxtová O., Lišková D., Kákoniová D., Kubačková M., Karácsonyi Š., Biliaics L.:
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14. Kubačková M., Karácsonyi Š., Lišková D., Kákoniová D., Auxtová O., Gallo J., Pôtoprstý V., Biliaics L.:
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15. Šubíková V., Slováková L., Farkaš, V.:
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16. Kolarova N., Grešík M.:
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21. Kremnický L., Alföldi J., Biely P., Tenkanen M.:
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23. Dupont C
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26. Mislovičová D., Šarovinová D., Kačuráková M., Stratilová E.:
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27. Gillet C., Liners F.:
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Plenary Lectures

STUDIES ON GLYCOPROTEIN GLYCANS

Johannis P. Kamerling
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In recent years the interest in carbohydrate (bio)chemistry has grown dramatically. This is mainly due to the general acceptance that carbohydrates and glycoconjugates exert essential biological and physico-chemical functions in a great variety of organisms.

In principal, progress in the studies on the carbohydrate chains of glycoproteins can only be made on the basis of a teamwork of structural analysis including conformational analysis, synthesis and biosynthesis of this type of molecules. The development of advanced methods to unravel the structures of the ensembles of carbohydrate chains present at specific amino acid residues in polypeptide backbones has been the first step in the glycoprotein glycan revival. Although nowadays several approaches based on ^{1}H-NMR spectroscopy (1, 2) and mass spectrometry (3) are available, and profiling techniques based on high performance separation procedures have come closer to non-carbohydrate chemists, the primary structure analysis of glycoprotein glycans has still not reached the level of a routine analysis. Due to the complexity of the carbohydrate chains, not only in terms of monosaccharide constituents and sequences, but also in terms of non-carbohydrate substituents, new more sensitive methods are needed. Even nowadays, completely novel oligosaccharide elements are identified. The knowledge of the specific oligosaccharide structures forms the basis for the investigations of their biosynthesis, and generates information concerning the glycosidases and glycosyltransferases involved. The (bio)chemical data can be of use for the remodeling of carbohydrate chains in recombinant DNA glycoproteins or for the (bio)synthetic preparation of biologically active carbohydrates. In addition, more advanced technologies will make it possible to study the three-dimensional structure of intact glycoproteins in high detail.

Using relevant examples of glycoproteins, like human α-chorionic gonadotropin, porcine zona pellucida glycoproteins, human recombinant erythropoietin, hemocyanins from *Lymnaea stagnalis* and *Helix pomatia*, stem bromelain, and the circulating anodic and cathodic antigens from *Schistosoma mansoni* worms, attention will

be paid to structural, biosynthetic and conformational aspects of both released and glycoprotein-bound glycans.

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New Approaches towards the Structural, Conformational and Dynamic Analysis of Biologically Relevant Oligosaccharides

S. W. Homans, Department of Biochemistry, University of Dundee, Dundee DD1 4HN UK.

There is currently great interest in the solution properties of oligosaccharides, in view of the fact that these moieties play a key role in a variety of biological recognition phenomena. Knowledge of their sequence, conformation and dynamics in solution may aid in the design of novel compounds which inhibit the recognition process, thus giving rise to drugs effective against various diseases states whose pathology is dependent upon such recognition.

Various experimental approaches involving high-resolution multidimensional, multinuclear NMR will be described for the determination of oligosaccharide conformation and dynamics in solution, both for free oligosaccharides and for the glycan moieties of glycoproteins. In particular, the question whether oligosaccharides are 'flexible' or 'rigid' in solution will be addressed by reference to NMR relaxation measurements. Finally, data will be presented regarding approaches for the determination of the solution structure of a carbohydrate-binding protein of a size (> 35 kDa) typical of many lectin-like protein domains.

DYNAMICS IN HEPARIN IN AQUEOUS SOLUTION STUDIED BY NMR RELAXATION MEASUREMENTS.

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Istituto Scientifico di Chimica e Biochimica "G.Ronzoni", via G.Colombo, Milano (Italy), *On leave from the Institute of Chemistry, Slovak Academy of Sciences, Bratislava (Slovakia)

The nature of overall and internal motions in the glycosaminoglycan heparin has been studied by NMR relaxation measurements. ^{1}H and ^{13}C NOEs, ^{13}C spin-lattice and spin-spin relaxation times have been collected at various magnetic field strengths. One-dimensional NOESY experiments, measured with three different mixing times, showed considerable large number of cross-relaxing protons within the monosaccharide units as well as across the glycosidic linkages. Due to the strong overlap of the ^{13}C resonances in the one-dimensional spectra, the ^{13}C relaxation times were measured by two-dimensional double INEPT. The relaxation rates were collected with and without suppression of cross-correlation effects between dipolar and chemical shift anisotropy relaxation mechanisms in order to estimate the influence of this effect on the relaxation data in this polysaccharide. Differences in the cross-relaxation rates between the protons relaxing through fixed distances and in the ^{13}C spin-lattice relaxation rates indicated that the molecule tumbles anisotropically in solution. The analysis of the data also showed that the effect of cross-correlation did not contribute more than 10% to the relaxation rates in heparin. The overall shape of the molecule was approximated with the model for a symmetric top and the rates of overall and internal motion as well as the order parameters have been estimated using a model-free approach.

POLYSACCHARIDE CONFORMATION AND NETWORK PROPERTIES

Edwin R. Morris

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The physical properties of disordered polysaccharides in solution depend predominantly on degree of space-occupancy (1). There is a sharp increase in the concentration-dependence of viscosity at a critical concentration (c^*) where the individual coils interpenetrate to form an entangled network. At concentrations below c^*, where the chains are free to move independently, viscosity (η) is virtually independent of shear-rate ($\dot{\gamma}$). Entangled networks of polydisperse chains (at $c > c^*$) exhibit (2) a general form of 'shear thinning' determined by the relative rates of disentanglement and re-entanglement:

$$\{\eta - \eta_s\} = \{\eta_0 - \eta_s\}/\{1 + (\dot{\gamma}/\dot{\gamma}_{½})^{0.76}\}$$

where η_s is the viscosity of the solvent, η_0 is the maximum 'Newtonian' viscosity at low shear shear-rates, and $\dot{\gamma}_{½}$ is the shear-rate required to reduce $(\eta - \eta_s)$ to $(\eta_0 - \eta_s)/2$. The value of c^* is inversely related to coil volume, as characterised by intrinsic viscosity, $[\eta]$. Plots of log η_0 *vs.* log $c[\eta]$ for most disordered polysaccharides superimpose closely (1), with $c^* \approx 4/[\eta]$, although some materials (notably galactomannans) have a lower c^* and steeper subsequent increase in η_0 due to specific associations between the chains.

Gelation occurs by formation of conformationally-ordered intermolecular junctions stabilised by co-operative arrays of non-covalent bonds (3) and, contrary to some suggestions in the literature, does not require the pre-existence of an entangled network. This is most clearly demonstrated by the capsular polysaccharide from *Rhizobium trifolii* (4), where the minimum critical gelling concentration (c_0) is about 60 times lower than c^*. Aggregation of ordered junctions can cause substantial thermal hysteresis between formation and melting of gel networks (3) and, for anionic polysaccharides, may be induced by non-specific charge-screening by simple electrolytes, incorporation of counterions within the aggregate structure, or site-binding of cations to the disordered polysaccharide coil. These three mechanisms are displayed by gellan gum (5) in its interactions with, respectively, $(CH_3)_4N^+$, Na^+ and Ca^{2+} ions.

Conformational ordering without development of a continuous network can generate characteristic 'weak gel' properties in solution. Gelation of welan and rhamsan (branched variants of gellan) after disruption and regeneration of the ordered conformation indicates that the weak gel properties of the native polymers (5) arise from perfect pairing of strands into uninterrupted double helices (6). Comparison of xanthan with related polysaccharides having longer or shorter sidechains (acetan (7) and xanthan polytetramer (8), respectively), suggests that conversion from 'weak gel' to 'true gel' properties in the presence of carob galactomannan or konjac glucomannan involves formation of heterotypic junctions between the cellulosic backbone of the xanthan molecule and unsubstituted regions of the mannan or glucomannan chain.

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On the relation between chemical structure and original solution properties of some bacterial polysaccharides

by M. Rinaudo, CERMAV, Grenoble (France)

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In this lecture, one intends to discuss the behaviour of some bacterial polysaccharides such as succinoglycan and hyaluronic acid ; these polymers have a wormlike chain behaviour. They are stereoregular polymers which for some of them adopt an helical conformation in well defined thermodynamic conditions. The helix-coil transition will be discussed and related with the polymer characteristics. These polymers are water soluble polymers and their main role is to increase the viscosity of the solvent. The influence of the molar mass, polymer concentration conformation and salt concentration on the viscosity will be introduced.

Polysaccharides such as gellan form gels in given conditions. The sol-gel transition is directly related with the coil-helix transition. The mechanism of formation of these thermoreversible gels will be discussed. The specific ionic selectivity will be demonstrated and its role on mechanical properties of the gel will be shown. The crosslink mechanism due to divalent counterions in the sol-gel transition of sodium polyglucuronate will be discussed and the properties compared with that of alginates or pectins.

Specific polymeric properties of polysaccharides are clearly directly related to their chemical structure.

PECTINS: CHEMICAL STRUCTURE AND CALCIUM-INDUCED GELATION

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Pectins are plant cell wall polysaccharides which contain a high proportion of galacturonic acids and which are used after extraction in food industry mainly as gelling agents. Their structure is characterised by a backbone of α-(1,4)-linked galacturonic acid residues, which are partly methyl-esterified, with some associated neutral sugars, typically L-arabinose, D-galactose, L-rhamnose (1). The polygalacturonic backbone may be interrupted by (1→2)-linked rhamnose; the frequency and the regularity of these interruptions probably have a profound effect on the gelation of pectins with calcium ions (2). Previous works based mainly on enzymic degradations of pectins led to the conclusion that they have long and regular uronic regions ("smooth") and rhamnose-rich regions ("hairy") carrying the other neutral sugars as side-chains (3).

We have studied (4) the length of the "smooth" regions, using the differences in susceptibility to acid hydrolysis of the glycosidic linkages. Linkages between uronic acid residues are notably more stable than those between an uronic acid and a neutral sugar and this leads to preferential cleavage of the linkages between galacturonic acid and rhamnose, thus liberating relatively whole the "smooth" regions. The length of the "smooth" regions is independent of the origin of the pectins, but the varying proportions of rhamnose (and other neutral sugars) shows that it is not the case of the "hairy" regions, in which some repeating units could also be found. The various pectins consist therefore of combinations of the same sub-units in different proportions. The beet pectins have a higher proportion of rhamnogalacturonic units in their "hairy" regions than the apple pectins, and than the citrus pectins (5).

The "smooth" regions, constituted by at least 100 monomers, can be associated through calcium ions, leading to gelation (6). The rôles of intrinsic parameters (their degrees of methoxylation, or amidation, substitution patterns), and of extrinsic parameters (concentration in pectins, pH, ionic strength, temperature) have been observed through phase diagrams (sol/gel/syneresis) and quantified using mainly specific electrodes (7, 8). The amount of bound calcium monotonously increases

whatever the physical state of the system and reaches a plateau, the value of which depending on the extrinsic parameters. In contrast, the concomitant release of the sodium ions is strongly effected by the phase transition. It was also shown (9) that the binding of calcium ions showed a cooperative character in the presence of external salt and an anticooperative character in water due to typical polyelectrolyte effects.

This study illustrates the rôles of extrinsic factors in the calcium-induced gelation of pectin and also allows to point out the importance of the stereospecificity in the binding of calcium ions to pectins.

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INTERACTIONS OF ANIONIC POLYSACCHARIDES WITH CATIONIC POLYPEPTIDES

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The interactions of some acidic polysaccharides (pectins, O-acetyl derivatives of pectate, alginates) with cationic polypeptides, mainly with polylysine enantiomers, have been studied. In the course of interaction the conformation of polypeptide changes from a disordered to a compact α-helical arrangement. Therefore, the complexation was investigated by circular dichroism measurement (CD). It was found that the interaction is of electrostatic nature and the complex-formation is governed by the stoichiometric ratio of charged groups of both interacting components. The decisive factor for the effective complexation is the conformation of polysaccharide in solution. In the case of pectate, polyguluronate and polymannuronate the interaction with polylysine enantiomers was found to be stereospecific, O-acetyl derivatives of pectate interact with both enantiomers. Moreover, the complexation efficiency is influenced by the degree of acetylation (DA); with increasing DA the efficiency of complexation decreases.

CHANGES IN POLYSACCHARIDES AND MECHANICAL PROPERTIES OF THE CELL WALL DURING AUXIN-INDUCED CELL EXTENSION

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It is generally accepted that auxin, either endogenous or exogenous, induces cell extension by changing the mechanical property of the cell wall, such as creep or stress relaxation, as measured by a variety of methods. The change in the mechanical property is brought about by biochemical modifications of cell wall components, particularly of matrix polysaccharides. The matrix polysaccharide composition is different between graminaceous and dicotyledonous plants. Beta-(1,3)(1,4)-glucans in the former and xyloglucans in the latter seem to be important in the regulation of auxin-induced changes in the cell wall and hence cell extension (4, 6). In the present paper the role of changes in xyloglucans in cell wall modifications in azuki bean (Vigna angularis) epicotyls will be discussed.

The three-step modifications at least, although overlapping, are involved in the changes in the cell wall polysaccharides, leading to cell extension; i.e. (A) degradation of xyloglucan molecules, (B) transglycosylation in xyloglucan molecules, and (C) synthesis of cell wall polysaccharides for continued growth.

Firstly, studies using antibodies against hepta- and octasaccharides of xyloglucans (3) and lectins, particularly fucose-binding lectins (2), as specific probes indicate that the breakdown of xyloglucan molecules is involved in auxin-induced cell wall changes. Secondly, the role of transglycosylation of xyloglucan

molecules in growth regulation has been suggested recently (1, 7), and an endo-xyloglucan transferase (EXT) has been isolated and purified from apoplast liquid of azuki bean epicotyls (8). Distribution of EXT proteins and EXT-mRNA has been studied in azuki bean epicotyls. The role of EXT in epicotyl elongation has been suggested. Thirdly, UDP-sugars are formed during auxin-induced growth and shown to be necessary for new cell wall synthesis which is required for continued cell extension due to auxin (5).

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CHEMICAL AND ENZYMATIC METHODS FOR THE RELEASE OF *N*- AND *O*-LINKED OLIGOSACCHARIDES FROM GLYCOPROTEINS

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It is now well established that oligosaccharides covalently attached to protein can have many roles including the ability to act as "molecules of intelligence" [1] and a dazzling variety of structures has been determined [2-4]. More recently, there has been increasing interest in the structures of oligosaccharides of recombinant glycoproteins of pharmaceutical interest [5-7]. This explosion of interest is attested to by the recent appearance of an international journal and a number of volumes devoted to methodologies in the area of Glycobiology [8-10].

Structural analysis of oligosaccharides generally requires their initial release from the glycoprotein backbone. Chemical methods include hydrazinolyis [11, 12] and β–elimination [13] for *O*-linked structures.

Enzymatic methods for release include the use of endo-β–*N*–acetylglucosaminidases (endo-D [14], endo-H [15] and endo-F [16]) which cleave the β1-4 linked di-*N*-acetylchitobiose core, while the Peptide-*N*^{4}-(*N*-acetyl-β-glucosaminyl) asparagine amidases (PNGase F [17] and A [18,19]) cleave the amide bond between the GlcNAc and Asn.

Endo-α-*N*-acetylgalactosaminidase D from *Diplococcus* [20] and A from *Alcaligenes* [21] are limited to the release of the disaccharide Galβ1-3GalNAc from Ser/Thr while that isolated from *Streptomyces* [22] is reported to release some additional, larger *O*-linked oligosaccharides.

The relative merits of the chemical and enzymatic methods will be discussed along with the substrate specificities of the various enzymes.

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GLYCOSYLATION OF PROTEINS IN BAKER'S YEAST AND WHAT IT IS GOOD FOR.

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The reactions initiating protein N-glycosylation are located at the endoplasmic reticulum and they are conserved from yeast to man. A precursor oligosaccharide attached to dolichyl pyrophosphate is transferred to nascent protein chains within the ER lumen. Subunits of a protein complex responsible for this transfer reaction have been identified and characterized recently by cloning the responsible genes (1-4). Gene disruptions have shown that protein N-glycosylation is vital for yeast cells (2,3), although it is not really understood why.

Protein O-glycosylation differs in fungal cells from that of higher eucaryotes. In baker's yeast the initial reaction proceeds in the ER and requires Dol-P-Man as sugar donor. The gene for the corresponding protein mannosyl transferase (PMT1) has been cloned and sequenced; gene disruption, however, did not turn out to be lethal (5). The null mutants were able to still O-glycosylate proteins *in vivo*, although at a reduced rate, indicating the existence of one or more further genes. In cooperation with H. Bussey's laboratory in Montreal the PMT2 gene has been found (M. Lussier et al., in preparation) and the gene product has been characterized (6). Recently evidence for two more PMT-genes in yeast has been obtained, as well as indications that a full knock out of protein-O-glycosylation is lethal.

To investigate to what extend saccharides of glycoproteins are of functional importance in cell-cell interaction, the a- and α-agglutinin, two cell surface glycoproteins of *S.cerevisiae* were purified, the genes cloned and the interaction of the gene products studied in detail (7). The data obtained indicate that sugar moieties do not play a major role in this specific cell/cell interaction process.

Hypothetical roles for protein N- and O-glycosylations will be discussed.

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BIOSYNTHESIS OF POLYPRENOLS AND THEIR GLYCOSYLATED DERIVATIVES IN ERGOSTEROL MUTANTS OF *S.cerevisiae*

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Selected thermosensitive, ergosterol auxotrophic mutants of *S.cerevisiae*, ie. erg mutants, served as a model to study biosynthesis of prenols and their glycosylated derivatives. Our preliminary experiments showed that in the two erg mutants i.e erg 8, blocked in the synthesis of mevalonic acid pyrophosphate and erg 9, blocked in squalene synthase, mannosylation of endogenous dolichyl phosphate was about four fold lower as compared to the parental strain. However, addition of exogenous dolichyl phosphate resulted in equal level of its mannosylation in both parental and mutants strains indicating that the anmount of endogenous dolichyl phosphate in the membrane praparations might be different in the mutants as compared to the wild type strain. Since we did not find differences in CTP-dependet phosphorylation of exogenous and endogenous dolichols/polyprenols between the strains we indirectly infer that the *de novo* synthesis of dolichyl phosphate in sterol mutants is impaired. Thus, in consequence activity of cis-prenyltransferase, the enzyme catalyzing condensation of isoprenyl pyrophosphate (IPP) to the allylic substrate - preferentialy farnesyl pyrophosphate (FPP) and most probably by subsequent addition of the IPP units, catalyzing synthesis of prenol pyrophosphates, was studied.
Still remains unclear why cis-prenyltransferase synthesizes a family of polyprenols of different chain length. We have established than *in vitro* the enzyme catalyzes synthezis of a family of free polyprenols of chain length between 10-16 isoprene units in the wild type strain and the mutants erg 8 and erg 9. Reaction is enhanced by addition of exogenous FPP. Mutants in FPP synthase i.e. erg 20 and the strain bearing erg20-2 gene on the multicopy plasmid under gal promoter (DD94), needed exogenous FPP for cis-prenyl

transferase to synthesize polyprenols *in vitro*. Surprisingly the DD94 construct besides normal size yeast polyprenols synthesizes prenologues of unusaual for yeast chain length up to 30 isopene residues.

Experimental work aiming to explain this phenomenon is currently going on.

TRANSFORMATION OF *TRICHODERMA* WITH YEAST MANNOSYL-PHOSPHODOLICHOL-SYNTHASE GENE LEADS TO THE INCREASED SECRETION OF CELLULYTIC ENZYMES

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It has been postulated that exoprotein secretion by the filamentous fungus *Trichoderma reesei* is related to their O-glycosylation. We have established that mannosyl-phosphodolichol synthase (MPD-Synthase) in *Trichoderma* like in yeast and other filamentous fungi is a key enzyme of O-glycosylation and its activity is modulated *in vivo* by the factors affecting protein secretion i.e. water soluble lipid precursors Tween 80 and choline, ethanol and temperature of cultivation.

To find out the more direct relation between the level of MPD-Synthase activity and cellulase secretion the TU6 strain of *Trichoderma* was transformed with yeast MPD-Synthase gene. Transformation resulted in the four to eight fold increase of MPD-Synthase activity when fungus was cultivated in minimal medium (ammonium sulphate the only nitrogen source). A heterologous expresion of the yeast gene was confirmed by the results of Western blott analysis and the results of immunoprecipitation of the enzyme activity.

The effect of the nutrient composition of the growth media on MPD-Synthase activity in the parental and transformed strain as well as on the one from *Saccharomyces cerevisiae* was compared. Addition of pepton or urea to the medium resulted in the increase of synthase activity in parental strain and remained without any effect on transformed one or on the enzyme from yeast. Among the factors known to increase MPD-Synthase activity and protein secretion only addition of ethanol to the growing medium or cultivation at

37°C affected positevely MPDS activity both in the parental and transformed strains. Taken together the activity of MPD-Synthase after transformation could be modulated by the growth condition of the fungus in a manner similar rather to the enzyme from *S.cerevisiae*.

The most striking result however, was 4-5 time increase of protein secretion by the transformed strain as compared to the parental strain. Since we didn't observed the direct effect of the introduction of the additional copy/copies of yeast MPD-Synthase on the overall level of O-glycosylation measured *in vitro* in the transformed *Trichoderma* cells, the molecular basis of this observation require further investigation.

EFFECT OF BREFELDIN A ON STRUCTURE OF *IN VIVO* AND *IN VITRO* SYNTHESIZED YEAST MANNANS

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Brefeldin A (BFA), a macrocyclic fungal antibiotic is known to inhibit effectively the secretory process in eucaryotic cells. The inhibitory effect consists in blocking the transport of proteins from ER to Golgi and redistribution of Golgi-located enzymes to the ER (1,2). In the yeast *Candida albicans*, the BFA inhibits the secretion of acid phosphatase and causes an accumulation of underglycosylated enzyme lacking the polymannose outer chain in the cytoplasm (3). We have investigated the effect of BFA on the activity of mannosyltransferases in the mannan synthase complex by analysing the structure of mannan synthesized both in vivo by intact cells and in vitro by membranes isolated from control and BFA-grown cells of *Candida albicans*. The results may be summarized as follows:

1. BFA itself at a concentration of up to 100 μg/ml has no effect on the activity of mannan synthase in the in vitro assay.
2. The membranes isolated from BFA-grown (14μg/ml) cells had 2 - 3 times higher specific activity of mannan synthase than membranes from control cells.
3. Mannans synthesized by membranes from BFA-grown cells had longer O-glycosidically linked mannooligosaccharides and longer N-glycosidically linked oligomannose side chains than mannans synthesized in vitro by control membranes.
4. There were no significant differences in structures of mannans isolated from intact control cells and from BFA-grown cells.

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A GTP-BINDING PROTEIN INVOLVED IN REGULATION OF YEAST CELL WALL BIOGENESIS

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In budding yeast, the biogenesis of the cell wall is strictly synchronized with the cell cycle, beginning at bud emergence and ending at daughter cell maturation. Controls must therefore exist to regulate the synthesis of cell wall constituents. The major structural component of the cell wall of *Saccharomyces cerevisiae* is a linear (1→3)-β-D-glucan that contains some (1→6)-β-linked branches. Synthesis of the linear polysaccharide is catalyzed *in vitro* by glucan synthetase, a membrane-bound enzyme that is highly stimulated by micromolar concentrations of GTP(1,2). By extracting membranes with salt and detergent, glucan synthetase from several fungi was dissociated into two fractions, A and B, both of which were needed for activity (3). We have now solubilized, by stepwise extraction, both fractions from *S. cerevisiae* and shown that A and B plus GTP are required for glucan synthetase activity. Purification of the A fraction led to the isolation of material that shows close correspondence between GTP-binding and glucan synthetase-complementing activity during chromatography. The most purified material (fraction A2) showed several bands upon SDS-polyacrylamide gel electrophoresis, but only the major band, at 20 kDa, was photolabeled by azido-GTP. A2 complemented B in the glucan synthetase assay, but GTP was no longer required. The requirement, however, returned when A2 was incubated with another fraction (A1), isolated during purification of A. In close correspondence with the increase in GTP requirement, GTP bound to the protein was hydrolyzed to GDP. Thus, A1 appears to act on A2 as a GTPase activating protein (GAP) in the same way as GAPs act on many other G-proteins belonging to the Ras superfamily (4). We conclude that A2 is a G-protein that regulates (1→3)-β-glucan synthesis

and may be regulated in turn by the GAP protein. These are probably the final steps of a regulating cascade that goes up all the way to cell cycle controls and acts to synchronize cell wall biosynthesis with the budding cycle.

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ROLE OF SACCHARIDES AND OLIGOSACCHARIDE DEGRADING ENZYMES IN PLANT DEFENSE SYSTEMS. James D. Anderson. Weed Science Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705 USA.

Oligosaccharides as well as simpler carbohydrates can play major roles in plants including defense against pest attack to regulating plant metabolism [1]. Some species produce specific carbohydrates that help protect them from insects, e.g., sucrose esters [2]; affect fruit ripening processes, e.g., oligosaccharide N-glycans [3]; or act as signals, e.g., pectic fragments, to induce hypersensitive-like responses [1]. Plants contain specific receptors for oligosaccharides which are known to induce such responses [4]. On the other hand, some solanaceous plants respond to the fungal (*Trichoderma viride*) cell wall digesting enzyme, xylanase, by inducing hypersensitive-type responses including ethylene [5], chitinase and glucanase [6] biosynthesis. The elicitation of such responses to this fungal xylanase does not seem to be caused by the production of biologically active oligosaccharides [7]. However, the symptoms induced by fungal proteins and oligosaccharides as well as other elicitors are very similar, e.g., ion movement, pH changes, phytoalexin production, gene transcription, cell death and tissue necrosis [5]. Possibly, some elicitors share a common signal transduction pathway that is recognized by different activated receptors. The mechanism by which xylanase-sensitive plants respond to this protein involves regulation by a single dominate gene [8] which

hasn't been reported for other elicitors. A model summarizing what is known about the action of xylanase is presented in Figure 1.

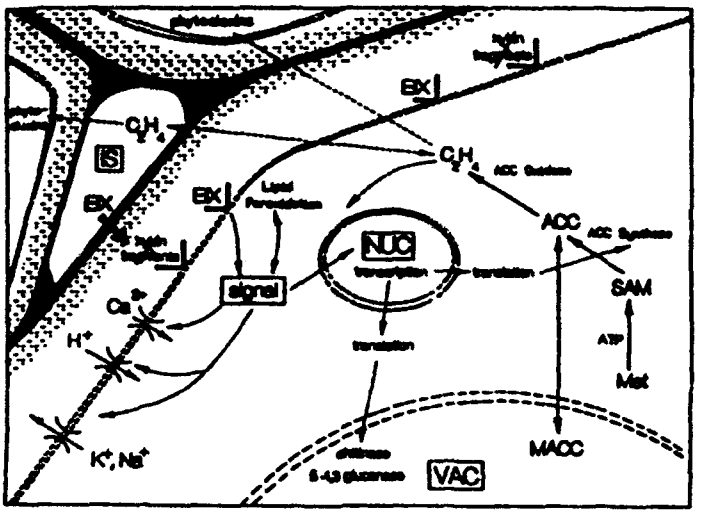

Figure 1. Proposed model by which a fungal xylanase elicits ethylene biosynthesis and other responses in Xanthi tobacco [5].

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EVOLUTION OF SUBSTRATE SPECIFICITY AND FUNCTIONAL DIVERSITY IN POLYSACCHARIDE ENDOHYDROLASES

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Comparative studies on the substrate specificities, three-dimensional conformations and structural features of corresponding genes provide compelling evidence that the (1→3)-β-glucan endohydrolases (EC3.2.1.39) and the (1→3,1→4)-β-glucan endohydrolases (EC 3.2.1.73) of higher plants have evolved along a common ancestral route. The evidence further suggests that the (1→3,1→4)-β-glucanase genes diverged from the (1→3)-β-glucanase gene family during the appearance of the graminaceous monocotyledons.

To identify catalytic amino acids in the two classes of enzymes, specific epoxyalkyl oligo-β-glucosides, carbodiimide labelling procedures and protein crystallographic data have been used to show that these catalytic amino acids are glutamic acid residues which occupy highly conserved positions in the primary sequences of the enzymes. Thus, the shift in substrate specificity has been achieved *via* a limited number of point mutations that have led to amino acid substitutions along a deep substrate-binding cleft that traverses the surfaces of the enzymes. The two catalytic amino acids are located approximately one-third of the way along the cleft and the distance of 8.2Å between the O^{ε} atoms is consistent with the proposed mechanism of hydrolysis, in which protonation of the glycosidic oxygen by the catalytic acid is followed by stabilization of the intermediate oxycarbonium ion by the catalytic nucleophile.

The evolution of new enzyme specificity enables a dramatic shift in function. The (1→3)-β-glucanases play a major role in protection of the plant against potential fungal pathogens, through their ability to hydrolyse the (1→3)-β-glucans of fungal walls. In contrast, the (1→3,1→4)-β-glucanases specifically hydrolyse plant cell wall (1→3,1→4)-β-glucans in the graminaceous monocotyledons during normal wall metabolism. Thus, one class of β-glucanase degrades fungal cell wall polysaccharides while the other degrades structurally-distinct polysaccharides of plant cell walls.

PURIFICATION AND PROPERTIES OF ALTERNANASE - A NEW TYPE OF α-1,3-α-1,6-GLUCANASE

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A newly isolated soil bacterium, tentatively identified as a *Bacillus* species, was found to be a constitutive producer of a novel type of glycanase that hydrolyses in an endo-fashion the polysaccharide alternan, an α-1,3-α-1,6-glucan, referred to in the literature as B-1355 dextran, which is produced from sucrose by enzymes of *Leuconostoc mesenteroides*. The glycanase, named alternanase, was purified from a D-glucose-spent culture fluid and has been characterized. The enzyme shows molecular mass of 100 000 and an isolelectric point 4.0. The enzyme shows maximum activity at pH 7 and the temperature of 40 °C. The enzyme is stable up to 50 °C and represents another α-glucanase that is inhibited by EDTA and activated by Ca^{2+} ions. The enzyme is specific for alternan. The rate and extent of hydrolysis of other α-glucans tested as substrates were negligible to those of the polysaccharide in which α-1,3-linkages alternate α-1,6-linkages. The enzyme shows relatively low specific activity on alternan when hydrolysis is followed by determination of reducing sugars. The reason for this finding is that the main hydrolytic products of alternan are non-reducing, and curiously enough, cyclic oligosaccharides.

ENZYMATICALLY PRODUCED CYCLIC α(1→3) AND α(1→6) LINKED OLIGOSACCHARIDES OF D-GLUCOSE

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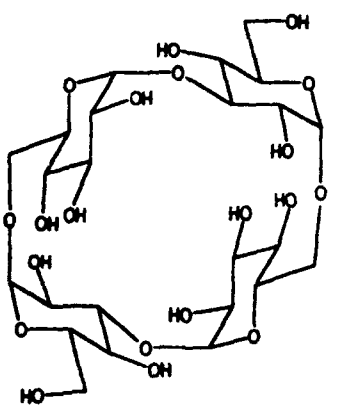
A new type of bacterial enzyme hydrolyzed alternan (*Leuconostoc mesenteroides* NRRL B-1355 fraction S dextran, an alternating α(1→3), α(1→6)-linked D-glucan), to give rise to a series of oligosaccharides. The oligosaccharide formed in the greatest proportion was a cyclic tetrasaccharide composed of D-glucose linked in an alternating α(1→3), α(1→6)-fashion (See Figure). Other saccharide products, formed in lesser amounts, included isomaltose and α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-D-glucose. Oligosaccharides of higher D.P. were also formed, including α-D-glucosylated derivatives of the cyclic tetrasaccharide. The structures of the oligosaccharides were determined by methylation analysis, chemical ionization mass spectrometry, NMR, FT-IR, partial acid hydrolysis, and enzymatic degradation. The tetrasaccharide has been crystallized, and has an $[\alpha]_D^{25} = +214°$. This is the first report of a naturally occurring cyclic tetrasaccharide.



INTERACTION BETWEEN XYLOGLUCAN AND CELLULOSE

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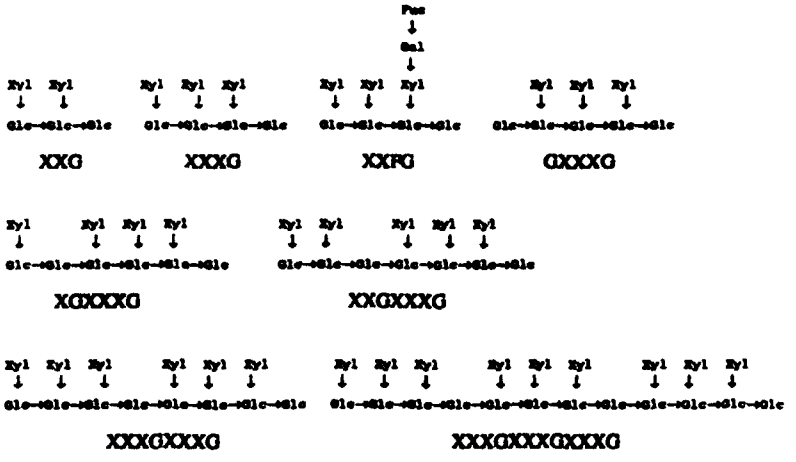
Binding of xyloglucans to cellulose microfibrils may occur specifically with structures that contain 1,4-β-glucosyl linkages in polymers with a conformation complementary to that of cellulose (1). Although the binding capacity of cellulose microfibrils is dependent on the surface of the binding area of the microfibrils, xyloglucans are not only partly embedded in but are also partly free between microfibrils, suggesting cross-link to cellulose microfibrils (2).

Xyloglucan oligosaccharides were isolated with various degrees of polymerization (DP) and reduced with tritiated sodium borohydride. The ^{3}H-oligosaccharides were tested for their ability to bind to amorphous and microcrystalline celluloses and to cellulose filter paper. Although neither XXG, XXXG and XXFG failed to bind to the celluloses, binding occurred with oligosaccharides with DP equivalent to more than four consecutive 1,4-β-glucosyl residues. The extent of binding to the celluloses increased gradually from GXXXG to XXXGXXXGXXXG, with the increase in the DP of 1,4-β-glucosyl residues.



Chemical structures of xyloglucan oligosaccharides

The binding of xyloglucan- and cello-oligosaccharides to celluloses can be expressed by a Langmuir adsorption isotherm, in which the levels of adsorption maximum are all similar but very low. Although an adsorption constant increased in the DP of the 1,4-β-glucosyl residues of xyloglucan- and cello-oligosaccharides, the adsorption constant of cellopentitol to cellulose was similar to that of XXXGXXXGXXXG, showing lower binding for xyloglucan oligosaccharides in spite of longer 1,4-β-glucosyl residues. The binding to cellulose of pea and *Tamarindus* xyloglucans also obeys a Langmuir adsorption isotherm, in which the level of adsorption constant with pea xyloglucan (150 DP of 1,4-β-glucosyl residues) is obviously higher than that with *Tamarindus* xyloglucan (3,000 DP). The level of adsorption maximum and adsorption constant of *Tamarindus* xyloglucan was decreased gradually from 3,000 DP to 64 DP of 1,4-β-glucosyl residues accompanying the decrease in the DP. This shows that fucosylated xyloglucan has a higher adsorption constant to celluloses than non-fucosylated xyloglucan at the same DP of 1,4-β-glucosyl residues. These findings indicate that xyloglucan binds to cellulose by mono-layer and fucosyl residues contribute to the increase of adsorption affinity.

Macromolecular complexes composed of xyloglucan and cellulose were produced by heating amorphous cellulose with xyloglucan in water at temperatures above 160 °C. The extraction of xylioglucan from the annealed specimens required concentrated alkali which might cause microfibrils to swell. Annealed specimens obtained by heating at 200 °C had a somewhat fiber-like appearance even though mixtures of amorphous celluloses and xyloglucan were completely amorphous before annealling. Annealing occurs specifically between amorphous celluloses at high temperatures, where xyloglucan may be entrapped into the bundles of cellulose fibers during fiber formation rather than bound to the surface of the fibers (3).

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ENGINEERING THE POLYSACCHARIDES OF THE PLANT CELL WALL MATRIX

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The cell walls of higher plants consist of a microfibrillar phase (cellulose) embedded in a matrix, which is composed mainly of polysaccharides. The mechanical properties of the plant primary cell wall determine to a large extent the textural properties of fruits and vegetables, and it is believed that wall mechanical properties are influenced, at least partially, by interactions involving the matrix polysaccharides. Much is known about the molecular structures of the main matrix polysaccharides in primary cell walls, yet very little information is available concerning the importance of particular structural features in determining wall mechanical properties. One approach to this problem is to seek to alter the structures of individual matrix polysaccharides in predictable ways within the cell wall of the living plant, and to observe any changes in tissue mechanical properties. This approach is being applied to the xyloglucan component of the plant primary cell wall. Hydrolytic enzymes with high degrees of specificity for xyloglucan are being isolated and their modes of action characterised. cDNA clones encoding these enzymes are being used to up- and down-regulate their activities in transgenic target plants. This talk will focus mainly on the

characterisation of specific, xyloglucan-acting enzymes, isolated from germinated nasturtium seeds.

In the nasturtium seed, a non-fucosylated xyloglucan is present in large amounts in the cotyledonary cell walls (1). It is a cell wall storage polysaccharide (2), and is mobilised after germination (1). Four hydrolytic enzymes have been shown to co-operate in the mobilisation of nasturtium xyloglucan (1): a xyloglucan-specific *endo*-(1→4)-ß-glucanase or xyloglucan endo-transglycosylase (3,4), a xyloglucan-specific ß-galactosidase (5), a xyloglucan oligosaccharide-specific α-xylosidase (6), and a ß-glucosidase. The modes of action of these enzymes individually on xyloglucan will be described, as well as their cooperative interaction in xyloglucan mobilisation. The isolation and characterisation of cDNA clones encoding these enzymes (7) will be described briefly.

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XYLOGLUCAN-DERIVED OLIGOSACCHARIDES AND THEIR EFFECTS ON ELONGATION GROWTH IN PEA EPICOTYLS.

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Xyloglucans of carrot cell walls were used as a source for oligosaccharides (XGOs) of defined structures. Some of these XGOs are biologically active. They inhibit the elongation growth of pea epicotyls in the zone 10 mm under the plumule hook when applied by the wound surface via the incubation medium. Ethylene is not induced by XGOs and consequently can be excluded as a signal transducer in our system. Transport studies with biologically active, tritium-labelled XGOs showed that the labelled material is transferred to the apex. This transport seems to take place with the transpiration stream.

Among the XGOs tested, XXFG (XG9) was the most effective compound. It inhibits NAA- and GA_3-stimulated elongation growth as well as "endogeneous" growth (1). Since the inhibitory effect is independent from the growth regulator applied to the sytem the XGOs seem to interfere with basic processes during cell wall loosening. In carrot cell cultures the XGOs confer no elicitor activity as can be demonstrated using PAL-activity and Ca^{2+}-influx as indicators.

Concerning the molecular mechanism of growth inhibition the effect of XGOs on wall-associated peroxidase isoenzymes was studied using carrot cell cultures and pea epicotyls. In an early response (XXFG) XG9 treatment enhances their catalytic activitiy dramatically. These results are in good agreement with the hypothesis of reinforcement of the wall by phenol-phenol and phenol-polysaccharide cross-linkages (2) leading to an enhanced stiffening and a reduction of elongation growth.

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RELATION BETWEEN STRUCTURE AND IMMUNOLOGICAL PROPERTIES OF THE POLYSACCHARIDE ANTIGEN OF *SALMONELLA TYPHI* (Vi)

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The capsular polysaccharide of Salmonella typhi (Vi) is a linear homopolymer of poly-α(1-4)GalpANAc variably O-acetylated at the C-3 position. Antibodies elicited by this antigen confer protection against typhoid fever (1). The relation between the immunologic properties and structure of Vi was investigated by carboxyl reduction, O-deacetylation. The immunogenicity of Vi was closely related to its degree of O acetylation. Partial O-deacetylation slightly increased immunogenicity; complete O-deacetylation eliminated the immunogenicity of Vi. Carboxyl reduction, in contrast, had a comparatively slight effect upon both the immunogenicity and antigenicity of Vi (2).

Immunogen (2.5μg)	OAc/Vi (% mol/mol)	Vi Antibodies in Mice (μg/ml, GM)
Vi	62	3.56
Vi-deOAc$_1$	45	4.39
Vi-deOAc$_2$	30	2.51
Vi-deOAc$_3$	1	<0.1
Vi-C$_{Reduced}$	43	3.43

The Courtauld-Koltun space-filling model of a pentamer of Vi demonstrated that the bulky nonpolar O-acetyls, which protrude in rows on both sides, make up most of the surface. The carboxyls are less exposed and are partially shielded by the O- and N-acetyls. The molecular model was further verified by binding studies with counter ions and bulky nucleophile (3). Potentiometric titrations

with K+ and Ca++ hydroxides showed that the difference in the free energy of binding between the two cations (ΔG^{Ca}_{K}) was inversely proportional to the degree of O-acetylation. Similar cationic effects were found when measuring circular dichroism.

Following the understanding of immunologic roles of the substituents on Vi, a semisynthetic vaccine was prepared from pectin (4). Pectin, a linear homopolymer of poly(1-4)-α-GalpA with <5% neutral sugars, was O-acetylated at both C2 and C3 positions to mimic the structure of Vi. The O-acetylated pectin (OAcPec) precipitated with antiserum against Salmonella typhi (5). However unlike Vi, the OAcPec was not immunogenic in laboratory animals probably due to its smaller size. OAcPec covalently conjugated with tetanus toxoid elicited Vi antibodies in mice and reinjection elicited a booster response.

Immunogen	Vi Antibodies in Mice (μg/ml)		
(2.5μg/injection)	1 Injection	2 Injections	3 Injections
Vi	0.65	0.76	Not Done
Vi-rEPA$_a$	0.85	17.1	12.7
OAcPec	<0.03	0.04	Not done
OAcPec-TT$_b$	0.98	5.47	6.29
OAcPec-TT$_c$	0.87	7.65	5.29

a,b,c are polysaccharide-protein conjugates

OAcPec has some advantages over the Vi because it can be measured by standardized colorimetric assays and also forms more soluble conjugates with proteins than Vi.

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STRUCTURAL FEATURES OF THE LIPOPOLYSACCHARIDE ANTIGENS OF COXIELLA BURNETII IN PHASE I AND PHASE II

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Q fever is a zoonosis caused by Coxiella burnetii, which is an obligate, intraphagolysosomal parasiting bacterium found worldwide. In C.burnetii, the lipopolysaccharide (LPS) is present as a structural component of the outer membrane of the cell envelope. In order to elucidate the biological function of C.burnetii LPS in relation to its structure, the LPSs from both virulent (LPS I) and avirulent (LPS II) phases are being investigated in detail for their composition and chemical structure.
The LPS II was found of the rough-type and contained D-mannose, D-glycero-D-mannoheptose, Kdo, and D-glucosamine in a molar ratio of 2:2:3:2, respectively. Based on the combined results of methylation-linkage analysis, FAB-MS, and ES-MS, a structural model for the LPS II has been proposed. In contrast, the LPS I is phenotypically smooth and has heterogenic O-polysaccharide chains. Two unusual sugars, namely virenose (6-deoxy-3-C-methyl-gulopyranose) and dihydrohydroxystreptose (3-C-(hydroxymethyl)-lyxofuranose) have been found in the LPS I in a considerable amount. Based on passive hemolysis tests with rabbit anti-phase I cell serum both sugars represent the immunodominant sugars of the LPS I. They are located in terminal positions of the O-polysaccharide chain.

ACTIVE SITES OF DERMATAN SULFATE FOR HEPARIN COFACTOR II. ISOLATION OF A NONASACCHARIDE FRAGMENT CONTAINING FOUR DISACCHARIDE SEQUENCES [α-L-IDURONIC ACID 2-O-SULFATE (1,3) β-D-N-ACETYLGALACTOSAMINE 4-SULFATE]

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Dermatan sulfate (DS) is a glycosaminoglycan with anticoagulant, profibrinolytic and antithrombotic properties, associated, at least in part, with its ability to inhibit thrombin by potentiating Heparin Cofactor II (HCII). The structure of DS is largely accounted for by the repeating disaccharide sequences [IdoA-GalNAc4SO_3], where IdoA is α-L-iduronic acid and GalNAc4SO_3 is N-acetyl-β-D-galactosamine 4-O-sulfate, linked 1,3 and 1,4, respectively. However, the activation of HCII is prevalently associated with minor, oversulfated sequences, which, in beef mucosal and pig skin DS, are prevalently [IdoA2SO_3-GalNAc4SO_3]. The longest of these sequences so far isolated as structurally homogeneous fragments contained three IdoASO_3 residues [M. M. Maimone and D.M. Tollefsen, *J. Biol. Chem.* 1990, 265, 18263-18271; G. Mascellani et al., *Biochem. J.* 1993, 296, 639-648].

Since the ^{1}H-NMR spectra of DS [V. Bossennec, M. Petitou and B. Perly, *Biochem. J.* 1990, 267, 625-630] show two signals (in the 5.2 ppm region) associated with H-1 of IdoA2SO_3 residues, the present work was addressed to quantify these residues, and identify the sequences in which they are incorporated. The content of IdoA2SO_3 of a number of DS of different origin was determined by integration of the corresponding H-1 signals in the 500 MHz spectra, as well as by HPLC analysis of disaccharides obtained by exhaustive cleavage with Chondroitinase ABC. Both sets of data showed a good correlation with the HCII-mediated inhibition of thrombin. Controlled Smith degradation of a beef mucosal DS afforded fragments which were separated by gel filtration on Ultrogel AcA 202 and further subfractionated by ion-exchange HPLC on Spherisorb 10 SAX. The structure of the longest of these fragments was characterized by HPLC analysis of digests with Chondroitinase ABC, mono- and two-dimensional NMR and mass spectroscopy. The fragment consisted mainly of the nonasaccharide GalNAc4SO_3 [IdoA2SO_3-GalNAc4SO_3]$_4$-R, where R is the remnant of a glycol-split uronic acid. Taken together with data on IdoA2SO_3 contents and NMR analysis of smaller oligosaccharides obtained in previous work, these results suggest that the relative area ratio of the two ^{1}H signals in the 5.2 ppm region essentially reflect the molar ratio between internal and "external" IdoA2SO_3 residues, i.e., the average lenght of the [IdoA2SO_3-GalNAc4SO_3] sequences.

ANTIMUTAGENIC EFFECTS OF GLUCANS

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Glucan, the component of most yeast and fungus cell walls, belongs to the naturally occurring agents with stimulating effects on the defense mechanism of the organism. Carboxymethyl derivatives of glucan (CMG) were shown to trap OH· radicals (1). The OH· radical is highly reactive and with respect to biological consenquences, it is regarded to be the most important radical (2). Since the trapping of free radicals seems to be one of the most promising approaches in the theory of antimutagenesis and anticarcinogenesis (3), we studied antimutagenic effects of glucans developed at the Institute of Chemistry, SASc, Bratislava. The results of the first experiments demonstrated the suppressing effects of three derivatives of CMG on the frequency of micronuclei (MN) in polychromatic erythrocytes (PCE) of A/Ph mouse bone marrow induced by 6.5 Gy of Co^{60} irradiation. In concordance with other authors (1 and 4), the derivative with the highest degree of substitution was found to be the most effective. The same suppressing effects of intraperitoneally (i.p.) or intravenously (i.v.) administered CMG derivatives were observed on cyclophosphamide induced frequency of MN in PCE in bone marrow of Swiss mice. The protective effects of CMG may be explained by their ability to trap OH· radicals and thus to decrease the clastogenic effects of mutagens. The results may be useful for therapeutic

application of glucans with radio- and chemotherapy. Due to its structure, sulfoethylglucan (SEG) was chosen to decrease the mutagenic effect of hexavalent chromium (CrVI). SEG administered i.v. or i.p. simultaneously with i.p. CrVI decreased the frequency of MN in PCE of ICR mice. Even more pronounced effect was achieved by SEG pretreatment 24 prior to CrVI administration. The mechanism of the protective effects of SEG could be explained either by the formation of Cr ion complexes with sulfoethyl groups of the glucan or by the scavenging ability of SEG to trap $OH^{\cdot}$ radicals. The results of our recent experiment with chitin-glucan (CHG) against CP mutagenicity, which proved to be effective only by i.p. and i.v. yet not by orally administered CHG, seem to indicate the failure of such high molecular weight molecules to pass through the gastrointestinal tract. All the presented results suggest a potentially beneficial effect of glucans used as antimutagens.

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Poster Session I

MOLECULAR MECHANICS AND SOLVATION ENERGY CALCULATIONS OF GLYCYL HEPARIN

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It had been previously reported that under alkaline conditions, heparin undergoes regio- and stereospecific epoxidation of α-L-iduronic-2-O-sulfate residues. A nucleophilic addition on heparin epoxide yields N-glycyl heparin, an anticoagulant agent. Its conformational behaviour may influence the interaction with membrane proteins and therefore the biological properties of glycyl heparin.

The conformations of models of the glycyl-derivative of glycosaminoglycan heparin have been studied by molecular mechanics calculations using the program CHAMP(93) (Conformational (Hyper) Analysis Milan Package, which was developed at I.C.M. C.N.R. by D. R. Ferro and M. Ragazzi). Net atomic charges which are necessary for calculation of the electrostatic contribution of the strain energy were assigned according previous results. Two-dimensional relaxed (complete optimization of all degrees of freedom during rotation) contour maps of the conformational potential energy as a function of the linkage glycosidic torsional angles φ and ψ were calculated. We examined also the influence of solvent effect on preferred conformers of glycyl heparin. However, for charged molecules the calculation of overall dipole moment is dependent on the origin of coordination system. To avoid this difficulty we used the procedure which shifted the origin of coordination system into center of mass of a molecule. Then the molecule was oriented in direction of its principal moment of inertia along the main axes. As well as quadrupole moment was calculated in translated coordinates.

THEORETICAL CONFORMATIONAL STUDY OF HEPARIN EPOXIDE IN AQUEOUS SOLUTION

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A biological function of oligo- and polysaccharides is linked with their three-dimensional structure (conformation). In order to interpret the experimental NMR data and to define the conformational properties of the polymer chain, a number of oligomeric models of heparin epoxide were studied by means of molecular mechanics. *Ad hoc* force-field parameters for the oxirane ring were included in the calculations.

Preliminary results indicated the preference of the $^5H_0$ ring form of 2,3-anhydro-α-L-guluronic acid residue over the alternative $^0H_5$ conformer. Moreover the crystal structure of the corresponding 1-methyl derivative is well reproduced by calculation. The same force field was used for an exhaustive analysis of two dimers, two trimers and one pentamer. Two dimensional (φ, ψ) maps of the dimers showed the location of the energetically favoured conformers; these were used as the starting points for building higher homologs. In agreement with the relaxation NMR data, the results indicate a significant flexibility of the polymer chain. The solvent effect on preferred conformers has been analyzed. The same charge distribution has been used as with molecular mechanics calculations of isolated molecule.

Supported by Alfa Wassermann S.p.A., Research Department, Bologna (Italy)

MOTIONAL PROPERTIES OF HEPARIN EPOXIDE IN AQUEOUS SOLUTION: NMR RELAXATION STUDY.

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^{1}H and ^{13}C NMR relaxation measurements at various magnetic fields have been used to characterize the nature of overall and internal motions in heparin epoxide in aqueous solution. Homonuclear two-dimensional NOESY experiment showed considerable large number of cross-relaxing protons in the molecule. The inter-proton distances calculated from NOE data were compared with those obtained by molecular mechanics calculations. Several discrepances between the experimental and the theoretical inter-proton distances as well as the variations in the ^{13}C spin-lattice relaxation times, measured at two magnetic fields, indicated that the polysaccharide tumbles anisotropically in solution. The rates of overall and internal motions as well as the order parameters have been calculated using a model-free spectral density function. The numerical values indicate that the correlation times which characterize overall molecular motion are outside the extreme narrowing limit ($\tau_{\perp} = 8\times10^{-10}$ s and $\tau_{\parallel} = 4.2\times10^{-8}$ s) and that internal motion correlation time is on a picosecond timescale.

FT-IR SPECTROSCOPY STUDY OF THE HYDRATION OF β-(1->4)-D-XYLANS AND RELATED OLIGOSACCHRIDES

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FT-IR spectra were recorded for xylan and related oligosaccharide models after equilibration at relative humidities between 76 and 98%, as well as for the titled carbohydrates in dilute aqueous solutions. Three ranges of wavenumbers (1550-1700), 1480-1200), and (1200-950) cm^{-1} corresponding to the vibrational modes of δ(OH), δ(CCH) and δ(COH), respectively, were investigated and the ratios of integrated intensities were established and found useful for differentiation of the behaviour of the studied samples. Correlations between the obtained results and the structural features of the studied molecules, including conformation, type and position of the interglycosidic bonds were found. The crystallization and hydration of the models was discussed in relation to intramolecular H-bond formation and to the ability to bound certain amount of structure water.

FT IR STUDY OF THE EFFECT OF HYDRATION OF THE STRUCTURE OF MONO- AND OLIGOSACCHARIDES IN AQUEOUS SOLUTION

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The nature and extent of conformational changes due to solvation in model systems were studied using FTIR-ATR spectroscpy. Infrared spectra of trehalose (α1->1), maltose (α1->4), melibiose (α1->6), sucrose (α1->2), raffinose (α1->6,α1->2), stachyose (α1->6,α1->6,α1->2), xylobiose (α1->4) and lactose (β1->6), as well as their constituent monosaccharides were recorded for aqueous solution in the whole range of concentration (5-80%, w/w) from dilute to saturated state.

The ratio of integrated intensities in the spectral regions, particularly sensitive to conformation modification, i.g. δ (COH) and δ(CH) vibrations (1180-950 and 1480-1200 cm^{-1}), respectively, is computed and correlated with concentration. The changes in band shapes show that effect of hydration derived from the spectral data is not linear in function of concentration but is rather conformationally dependent. The sugar hydration which is affected by the number of equatorial hydrogen groups [1] can be followed by IR spectra as well as the role of the CH_2OH group and of the ring size.

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COMPARATIVE INTERACTIONS OF MAGNESIUM AND CALCIUM COUNTERIONS WITH POLYGALACTURONIC ACID

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The results of the study of comparative interactions of magnesium and calcium counterions with carboxyl groups of polygalacturonic acid employing potentiometry, conductimetry, ultracentrifugation, and circular dichroism are presented. The measurements have been performed in salt free solutions. It can be concluded from the results obtained that binding of these divalent counterions is of different nature. While magnesium counterions interact with polygalacturonic acid electrostatically without any cooperativity, in the case of calcium counterions the strong interchain cooperative interaction associated with a conformational change of polygalacturonate chain is again demonstrated.

ISOLATION AND CHARACTERIZATION OF THE EASILY EXTRACTABLE POLYSACCHARIDE COMPONENTS OF *Cistanche deserticola* Y.C.Ma

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Cistanche deserticola Y. C. Ma. is a holoparasite growing on the roots of the small tree *Haloxylon ammodendron* which is widely distributed in the Gobi desert. Pharmacological studies carried out by a Mongolian group have shown that the decoction of the *Cistanche* herba exhibits anabolic, antistress and adaptogenic activities. As the last mentioned properties might be localized in some of the carbohydrate components of the drug, polysaccharide fractions have been isolated from the subterraneous part of *Cistanche* herba by a sequential extraction procedure using methanol, cold water, hot water, 0.5 M NaOH, and 0.1 M EDTA. The isolated polysaccharides were fractionated by CTAB - precipitation, gel and anion-exchange chromatography. Based on the sugar composition, methylation analysis and NMR spectroscopic data, it was shown that the bulk of the easy extractable polysaccharide fractions is composed of α-1,4-D-glucan, L-arabino-D-galactan, 4-O-methyl-D-gluc-urono-D-xylan and pectic polysaccharides. Structural features, suggested to be involved in various immunological activities[1], have been found in some of the above mentioned fractions.

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p-CARBOXYBENZYL DERIVATIVES OF D-XYLANS: SYNTHESIS AND STRUCTURE

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The knowledge of the molecular properties of xylans is of fundamental importance not only for practical applications of these biopolymers, but also for the understanding of their physiological role in plant and human organisms. Most of the isolated xylans show a linear, low-branched backbone and, therefore, a high density of hydrogen bonds in their solid state. These features are suggested to be responsible[1] for the low solubility of xylans in water and classical solvents what limits their practical applications and causes troubles in the characterization of the molecular properties.

To overcome the solubility problems, *p*-carboxybenzylation of the most abundant structural types of xylans under mild reaction conditions has been investigated. The new polymers have been fully characterized using UV, IR and NMR spectroscopy, and potentiometric titration. Degradation of the polymer due to derivatization was tested by HPGPC and viscometry. The incorporation of UV-absorbing substituents into the molecular chains may be advantageous for molecular weight determination and interaction studies of xylans by use of UV-scanning ultracentrifuge.

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SOME PROPERTIES OF QUATERNIZED HETEROXYLANS

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Xylans amount to a third or more of hardwoods and annual plants and represent a potential biopolymer source for technical and other applications[1]. Chemical modification of xylans enables us to increase their utilization possibilities. For this purpose, we have prepared trimethylammonium-2-hydroxypropyl (TMAHP) groups containing heteroxylans[2] with a different macromolecular backbone. The distribution of the TMAHP substituents of these derivatives were characterized by NMR spectroscopy. HPGPC, viscometry and light- scattering were used to study the solution properties. The results were discussed in relation to the adhesive and flocculating ability of quaternized xylans, utilizable in papermaking[2]. The significant antimicrobial activity of quaternized xylans against some gram-positive and gram-negative bacteria, depending on the xylan structure and degree of quaternization[3], may be influenced also by the solution properties of these derivatives and could be useful in medical applications. The derivatives are degradable by yeast-like microorganisms[4].

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CHEMICAL MODIFICATION AND FRACTIONATION OF PEA STEM POLYSACCHARIDES

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Annual plants represent an important source of polysaccharides. The primary cell wall composition of these polysaccharides differ from secondary cell wall. The main difference is in the presence and quantity of pectic and xyloglucan polysaccharides, protein content and degree of lignification. Pea (Pisum sativum L. cv. Tyrkys) stems represent a good source for the study of fractionation methodologies of the primary cell wall polysaccharides. It is known that chemical procedures degrade pectin and hemicellulose polymers more dramatically than enzymatic methods. The less common method in fractionation procedures is chemical modification of plant cell walls with chemical group which could change separation features of its components. In our previos studies of wood materials and annual plants we have used quarternary ammonium groups for modification and extraction of plant cell wall.

In the present work we have isolated polysaccharides by direct chemical modification of pea stems and compared these fractions with materials isolated by known methods. Quarternization of the material in situ results in isolation of modified polygalacturonic acid. This water-soluble fraction did not contain peptides in contrary to sample treated with pronase, amylase and ammonium oxalate. From the later sample a mixture of oligomers of GalA was isolated containing also some Araf side chains. The pretreatment with sodium periodate gave water-soluble galactoxyloglucan which was partially oxidized. The direct extraction of pea stems with water gave predominantly peptides while with 6M KOH water-soluble galactoxyloglucan fraction which contained 1.4% of nitrogen (protein residues) was obtained.

CHARACTERIZATION OF LIGNIN-CARBOHYDRATE COMPLEXES FROM THE WOOD HOLOPARASITE *Cistanche deserticola* Y.C.Ma.

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Chemical analysis of *Cistanche deserticola* which parasites on the root of the angiosperm *Haloxylon ammodendron* L. showed the presence of lignin component in the amount of 6.4%, that was not characterized before. Different lignin-carbohydrate fractions have been isolated by dioxane-water extraction of the methanol pre-extracted and depectinated Cistanche plant as well as by successive extraction of the obtained residue with water and 4% aqueous NaOH solution in the yields of 0.1-0.7%. In addition, a lignin fraction was prepared by acidolysis of the methanol pre-extracted plant in dioxane in the yield of 12%. All fractions appear significantly rich in non-lignin constituents - carbohydrates, phenolic acids and proteins. The latter amounted 3-20% of the fractions.

Using ^{13}C NMR-, UV-, and IR spectroscopy and sugar analysis, it was shown that the isolated fractions represent lignin-carbohydrate complexes with various lignin to carbohydrate ratios. Moreover, the results obtained indicate that the lignin moiety consisted of guaiacyl-, syringyl-, and p-hydroxy phenyl units. Therefore, the Cistanche lignin could be considered to be a typical gramineous plant lignin. It contains fewer β-aryl ether linkages than lignin of hardwoods to whom the host plant belongs.

^{13}C NMR STUDY OF SOLID-STATE REACTION OF CELLULOSE WITH LIGNIN MONOMERS.

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Interaction between cellulose and lignin during thermal treatment of lignocellulosic materials has been investigated by ^{13}C NMR spectroscopy of the products obtained by solid-phase reaction of cellulose with lignin model compounds. High resolution NMR spectra of the product of reaction between vanilyl alcohol and cellulose, dissolved in the system of dimethyl sulfoxide - N-dimethyl morpholine at 90°, indicated that the linkage between lignins and polysaccharides could be formed. Since the product obtained from the reaction of veratryl alcohol was not soluble in this solvent, the formation of the carbohydrate-lignin bond has also been investigated by ^{13}C CPMAS NMR spectroscopy. The spectra were found to be comparable with those recorded for lignin-saccharides complexes in native lignocellulosic materials. Proton relaxation times were measured during a spin-diffusion experiment from the point of view of each phase; this made possible to recover the magnetization on ^{13}C nuclei under high resolution conditions. The lignin monomers and the amorphous phase of cellulose showed the same relaxation times indicating that they are mixed on a scale of ten nanometers. This result is consistent with the existence of lignin-carbohydrate linkage formed secondary during the thermal treatment of wood.

A NEW METHOD FOR SYNTHESIS OF ESTER LIGNIN-SACCHARIDE MODEL COMPOUNDS

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The nature of interactions between lignin and polysaccharides in plant tissues has been examined extensively in our recent papers by the isolation of soluble lignin-carbohydrate complexes as well as by using of model compounds representing acetal, glycosidic and ether types of linkage. The subject of the present work is synthesis of p-methoxybenzyl ester D-glucuronic acid.

As to low stability of p-methoxybenzyl ester linkage in p-OCH_3benzyl 1,2;3,5-di-O-benzylidene-α-D-glucofuranuronate during removal of benzylidene groups by hydrogenolysis and/or acid hydrolysis anisylidene groups were used for blocking of free hydroxyl groups of glucuronic acid instead of benzylidene derivatives. The possibility of their replacement by acetals derived from anisaldehydes resulted from our previous observations (1,2), that the latter have 100 times high lability towards acid hydrolysis compared to benzylidene derivatives.

p-OCH_3 Benzyl 1,2:3,5-di-O-anisylidene-α-D-glucofuranuronate was prepared by electrofilic reaction of N,N-dimethylformamide dineopentyl acetal and p-methoxybenzyl alcohol with di-O-anisylidene derivatives of D-glucuronic acid. Neopentyl acetal would not esterify acid (3) however it enter into acetal exchange reaction with anisalcohol, resulting in esterification of glucuronic acid. p-Methoxybenzyl D-glucuronate was obtained by following mild acid hydrolysis (70% acetic acid, 60°C for 6hod) quantitatively.

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STRUCTURE AND PROPERTIES OF FILMS PRODUCED FROM METHYLCELLULOSE AQUEOUS GELS

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Methylcellulose macromolecules are a statistic block copolymer consisting of hydrophilic and hydrophobic fragments of different rigidity (1).
Formation of the methylcellulose film structure has been studied in the present work using methods of X-ray phase, differential thermal and thermomechanical analyses. The polymer structure was modified by directed changing the hydrophilic/hydrophobic balance under thermokinetic effects.
Two types of interactions are realized in the films produced from methylcellulose aqueous solutions by the heterogeneous method that is shown as two reflexes on diffractograms of samples. Annealing at 200°C leads to increasing the number of ordered structures formed with the participation of both methoxyl and hydroxyl groups, intensity of both reflexes being increased 2-3 times.
Structure formation in films produced from a gel has been explained from the position of microphase separation when gelling in a methylcellulose-water system. In this case formation of an ordered structure occurs as the result of orientation through macromolecule hydrophobic areas. Relative reflex intensity increases 10 times compared with samples from an aqueous solution.

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COMPARATIVE STUDY ON ORGANIC ACIDS FERMENTATION BY CELLS ENTRAPPED IN CALCIUM PECTATE AND CALCIUM ALGINATE GEL BEADS

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Calcium pectate gel (CPG) is an alternative to calcium alginate gel (CAG), which is probably the most used polyanion for immobilization of biocatalysts by entrapment. Molecular, morphologic, diffussional properties of CPG and CAG are very similar (1), some advantage due to higher stability of the calcium pectate salt was expected. This paper presents comparative study on the mechanical properties of CPG and CAG and biotechnological parameters on organic acids fermentation by cells (*G. oxydans*, *A. niger*, *R. arrhizus*) entrapped in CPG and CAG beads. It is shown that CPG beads are more stable against Ca^{2+}-complexing reagents, but this advantage is depressed during a fermentation of organic acid due to the pectolytic activity of used microorganism. The operational stability of cells entrapped in CPG beads is higher than of those entrapped in CAG beads.

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CONCANAVALIN A - INVERTASE AFFINITY CHROMATOGRAPHY. MONITORING BY ENZYME FLOW MICROCALORIMETRY.

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A specific sorbent for glycoenzymes from bead cellulose with covalently bound Concanavalin A (Con A) was prepared by using the trichloro-s-triazine method. This conjugate was evaluated as a carrier for affinity chromatography of invertase. The optimal conditions for specific adsorption and desorption were estimated taking into consideration extraordinary high stability of invertase : Con A-cellulose complex (1). The chromatographic tests on small columns were used to find appropriate conditions : the equilibration acetate buffer pH 4.7 with 0.1 M NaCl; less than 1.5 mg of immobilized Con A per 1 ml of the gel; the incubation period for releasing of bound invertase with specific eluent α-methyl-D-mannopyranoside.
In recent papers (1,2) was described the possibility to determine the amount of invertase immobilized on the carrier by a flow microcalorimetric method. This direct method (applying the linear relationship between content of the bound enzyme and ΔT_{max} value) was utilized as the probe to verify the amount of invertase adsorbed to the carrier in any stage of the affinity chromatography experiment.

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METHODICAL ASPECTS OF CHARACTERIZATION OF PECTATE SALTS BY SIZE-EXCLUSION HPLC ON SEPARON HEMA BIO 1000 COLUMN PACKINGS

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The reliability of both light scattering (1) as well as size-exclusion (SE)-HPLC depends on the complete removal of extraneous matter from the polymer solution. The use of membrane filter or ultracentrifuge (1,2) is common practice, but in the case of pectate salts satisfactory results have not been provided yet. This paper introduces the preparative separation by GPC on Sepharose columns as an alternative method which should diminish the heterogeneity of pectate. Efficiency of such a separation of molecularly dispersed pectate(s) from extraneous material is discussed. Moreover, calibration of the SE-HPLC by means of narrover unimodal fractions of pectate is presented. It may be confirmed (3) that the universal calibration relation is not always valid in aqueous SE-HPLC and the validity has to be verified for each system under consideration.

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CHARACTERIZATION OF BIOSCPECIFIC SORBENTS BY THERMAL ENZYME-LINKED BINDING ASSAY (TELBA)

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The preparation and characterization by thermal enzyme-linked binding assay of biospecific sorbents for glycoprotein-affinity chromatography is described. The lectin Concanavalin A (*Canavalia Ensiformis)* isolated from Jack Beans is used as a natural ligand to recognize a non-reducing manoside terminal of glycoproteins. The Bead Cellulose (Perlose MT-100, particle diameter 100-200 μm, dry weigh 12.5 %) is a non-soluble support with standard spheric surface. The various methods for covalent coupling of Con A to Bead Cellulose is described. Among these methods the activation of Bead Cellulose by cyanuric chloride offer the biospecific carrier with the highest amount of covalently linked Con A (29.97 mg/g). Invertase (β-D-fructofuranosidase, E.C. 3.2.1.26) from bakers yeast (specific activity 100 $U.mg^{-1}$) is used as a reporter enzyme to characterize the biospecific sorbent (Con A-cel). The method to characterize of Con A-cel is based on the calorimetry measurement of temperature changes of enzymic reaction of the linked appropriate enzyme (invertase). The base of the "software" (mathematical model) to transform the measured thermometric signals (ΔT) into kinetic constants was established previously (1, 2). This work shows the "hardware" (calorimeter so-called Enzyme Thermistor) adapting to measure absolute kinetic constant directly by autonomy flow-through calorimetry system omitting postcolumn analytical method.

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PARTITION MECHANISM OF PROTEIN ADSORPTION ONTO BEAD 2-HYDROXY-3-PHENOXYPROPYL CELLULOSE. ROLE OF EXTERNAL SURFACE OF CELLULOSE PARTICLES.

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Adsorption of proteins onto 3-phenoxy-2-hydroxypropyl derivatives of bead cellulose (PHPC) was studied. Partition coefficient p characterizing the material balance of adsorption in equilibrium was found to show a tendency to increase with increasing of protein molecular weight. Affinities of protein - ligand interactions were found to follow a similar tendency because apparent dissociation constants of these interactions (K_D as a reciprocal parameter) decreased with increasing protein molecular weight. Thus, size exclusion properties of the original bead cellulose did not control adsorption onto hydrophobized PHPC. Fluorescence microscopy of PHPC particles after adsorption of fluorescein (as an amphiphilic fluorescent label) revealed localization of the label on the external surface of cellulose particles. The 3-phenoxy-2-hydroxypropyl- (PHP-) groups should be localized predominantly at the external surface of cellulose particles too, accordingly, steric problems of fluorescein (as a low-molecular-mass label) in penetration into the internal space of bead cellulose macroporous structure are improbable. Open microenviroment around the PHP-groups localized on the external surface of cellulose particles could not be an effective steric barrier in the contact with protein molecules. This may be considered as explanation for the lack of size exclusion properties in the control of protein adsorption onto the PHPC.

ENHANCED PROCEDURE OF METHYLATION ANALYSIS.

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The most widely used method for determination of linkage structure of polysaccharides and glycoconjugates is methylation analysis by GLC of partially methylated alditol acetate derivatives of sugars. Mass spectrometry has become an important and versatile technique in carbohydrate chemistry for identification of products of methylation analysis. From EI mass spectra one can deduce the original distribution of the methoxylated and acetoxylated carbons on the carbon skeleton. In this regard, the special database of EI mass spectra of nonreducing terminal, linked, branched, unmethylated, trideuteriomethylated and underacetylated alditol acetate derivatives of sugars has been created. But one must also bear in mind that not only will sugar derivatives be separated chromatographically, but many contaminants as well. Hence, one derivative may be hidden in the spectrum if the other predominates. Chemical ionization MS can be used to generate molecularś ions. In our work new method for CI MS of products of methylation analysis has been developed using protonated pyridine as reaction agent.

The major advantage of this method is that the obtained spectrum contains molecular weight information in the form of only one pseudomolecular ion species. This is the reason for rapidly increasing of sensitivity unlike the other CI and EI methods. The molecular weight data derived from cluster ions provide information on the type of the molecule and usually reveals the presence of any additional noncarbohydrate components.

TRANSFORMATION REACTION COURSES OF D-GULOSE AND D-IDOSE

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D- Gulose and D- idose undergo conversions, which are different for both of them and depend on reaction conditions. The courses of transformation in acid medium has been monitored by HPLC and GC - MS , paper chromatography and DC- polarography[1-3]. The catalytic effect of molybdate ions in water solutions at pH 3.1 and 5.1 has been observed. These conversions have been performed at conditions optimized experimentally. Reaction activity of both saccharides, participation in epimerization, isomerization and dehydration reactions, character and yield of reaction products has been observed and compared with conversions in acetate buffer (pH 4.6) at the same conditions.

Both aldohexoses at molybdate ions catalyzed transformations prefere mutual epimerization. Isomerizations and dehydrations take places as well. Gulose is more stabile than idose. Epimerizations give higher yield in ammonium molybdate solutions. On the other hand isomerizations and dehydrations give higher yields in molybdic acid. Evidently obtaining equilibrium of aldoses in solution plays an important role. Gulose is stabile and idose is dehydrated in acetate buffer.

Ketohexoses are formed from both aldoses in the some medium of molybdate ions at the same ratios as well:90 % sorbose, 8 % fructose and maximum 2 % psicose and tagatose calculated on the over-all products of isomerizations. Analogically deoxysaccharides as products of dehydration reactions are formed. Generally, products with 2/3 of one deoxy group and 1/3 with two deoxy group are formed. Non-saccharide type of compounds namely furaldehyde derivatives have been detected with maximum concentration of 1 %.

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SYNTHESES, STRUCTURE, AND CONFORMATION OF SOME N-ACETYL--GLYCOSYLAMINES AND N-ACETYL-DIGLYCOSYLAMINES

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N-Acetyl-bis(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amine (I) was prepared by N-acetylation both of (2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amine and bis(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)amine, respectively. Deacetylation of compound I gave N-acetyl-di-β-D-glucopyranosylamine. N-Acetyl-bis(2,3,4-tri-O-acetyl-β-L--rhamnopyranosyl) amine (II) was prepared by N-acetylation of bis(2,3,4-tri-O-acetyl-β-L-rhamnopyranosyl)amine. Deacetylation of compound II gave di-β-L-rhamnopyranosylamine.

The structure, anomeric configuration, and conformation of these compounds as well as of N-acetyl-β-D--xylopyranosylamine and N-acetyl-2,3,4-tri-O-acetyl-β--D-xylopyranosylamine were determined by ^{1}H and ^{13}C NMR spectroscopy.

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TRANSGLYCOSYLATION REACTIONS OF SOME GLYCOSYLAMINES

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Di-β-D-galactopyranosylamine has been prepared by transglycosylation of β-D-galactopyranosylamine. By treatment of 4,6-O-ethylidene-α-D-glucopyranose with ammonia 4,6-O-ethylidene-β-D-glucopyranosylamine was prepared. The structure, anomeric configuration and conformation of these compounds as well as of N-acetyl-β-D-glucopyranosylamine, N-acetyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine, and N-acetyl-β-D-mannopyranosylamine were determined by ^{1}H and ^{13}C NMR spectroscopy.

The results of the study indicate that the favored position of the anomeric amino group of aldosylamines and dialdosylamines is equatorial. This is in agreement with the fact that the expected anomeric effect would be small with these structures and that any positive charge on the nitrogen atom would furthemore result in an inverse anomeric effect, with increased stabilization of the anomeric group in equatorial orientation.

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O-(3-ALKYLTHIO-2-HYDROXYPROPYL)SACCHARIDES - PREPARATION, CHARACTERIZATION AND SOME PROPERTIES

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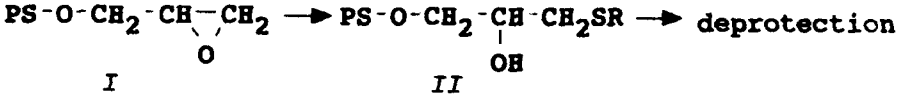
Some alkylthiosaccharides are known as non-denaturing detergents for the solubilization and reconstitution of membrane proteins (1).

Mono-epoxypropylated derivatives (*I*) prepared from suitably protected mono- and oligosaccharides (PS-OH) when reacted with alkanethiols (alkyl = n-hexyl, heptyl, octyl, decyl, dodecyl), afforded corresponding sulfides (*II*).



After deprotection obtained *O*-(3-alkylthio-2-hydroxypropyl)saccharides exhibited surface activity and some of them (when alkyl = n-octyl) exhibited also moderate antimicrobial activity against some gram-positive bacteria.

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PREPARATION OF SOME CARBOHYDRATE GLYCIDYL ETHERS

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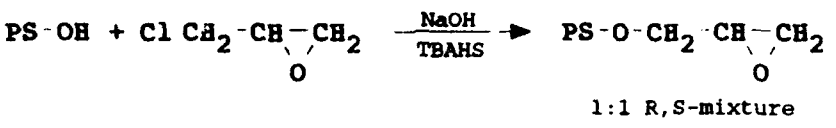
Carbohydrate glycidyl ethers represent a suitable substrate for the preparation of immobilized carbohydrates, widely used in many areas of chemistry and biochemistry. Reaction products with biopolymers are of high interest as potential carriers of drugs and as a model in the study of protein-carbohydrate-interactions. Epoxyalkyl glycosides have been investigated as enzyme inhibitors (1).

We have described (2) a high yielding, one-step method for the preparation of the title compounds starting from suitably protected saccharide (PS-OH), epichlorohydrin and phase transfer catalyst.



In some cases, we were successful to separate pure diastereoisomers from the 1:1 reaction mixture by the simple repeated crystallization.

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Poster Session II

A NEW APPROACH TO C-GLYCOSIDES OF PURINES

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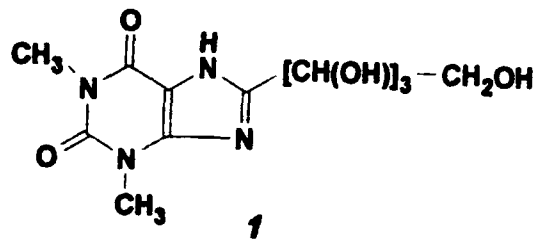
The C-glykosides of purines have been hitherto synthesized by oxidation of polyacetoxyalkylazomethines prepared from 5,6-diamino-2,4-(1*H*,3*H*)-pyrimidinedione and pentoses or hexoses. The conversion of the synthesized 8-(polyhydroxyalkyl)purines was not studied.

As continuation of our previous studies of 8-substituted alkylpurines we now report another approach to synthesis of 8-(1,2,3,4-tetrahydroxybutyl)-1,3-dimethyl--1,2,3,4-tetrahydro-7*H*-purine-2,6-diones (1)



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starting from 6-chloro-1,3-dimethyl-2,4-(1*H*,3*H*)-pyrimidine (2). Aminolysis of this 6-chloro derivative 2 with an aqueous solution of 1-amino-1-deoxypentitols afforded 6-(2,3,4,5-tetrahydroxypentylamino)-1,3-dimethyl-2,4-(1*H*, 3*H*)-pyrimidinediones (3). Acid catalyzed C-nitrosation of the latter gave the corresponding 5-nitroso derivatives 4, which on reflux in organic solvents yielded the required compounds 1. Their structure was verified by ^{1}H and ^{13}C NMR spectra.

STRUCTURE OF THE O-SPECIFIC POLYSACCHARIDE OF *PROTEUS PENNERI* 35 CONTAINING 2-ACETAMIDO-4-*O*-[(*S*)-1-CARBOXYETHYL]-2-DEOXY-D-GLUCOSE

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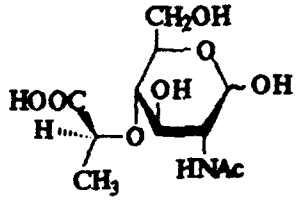
Proteus bacteria are opportunistic human pathogens which cause urinary tract infections. We studied the structure of an acidic O-specific polysaccharide of a new species *P. penneri* strain 35. Its acidic component was isolated by solvolysis with anhydrous HF followed by anion-exchange chromatography and identified as 2-acetamido-4-*O*-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucose (**1**). It was indistinguishable by ^{1}H and ^{13}C NMR spectra, retention time, and specific optical rotation from the authentic sample synthesized by alkylation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside with (*R*)-2-chloropropionic acid followed by hydrogenolysis over Pd/C.



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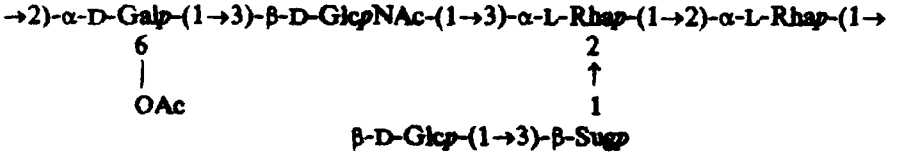
On the basis of ^{1}H and ^{13}C NMR study, including 1D NOE spectroscopy and 2D homonuclear and heteronuclear $^{13}C,^{1}H$ COSY, the following structure of the O-specific polysaccharide was established:



where Sug is the residue of **1** and the degree of *O*-acetylation is ~40%.

A RHAMNOARABINOGALACTAN FROM THE LEAVES OF *PLANTAGO LANCEOLATA L., VAR. LIBOR*

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The biologically active mucilage isolated from the leaves of *Plantago lanceolata L., var. libor* has been fractionated by anion-exchange and gel permeation chromatography to give a neutral polysaccharide composed of L-rhamnose, L-arabinose, and D-galactose in the mole ratio of 0.21 : 0.66 : 1.00. Structural studies performed by glycosyl linkage composition analysis, ^{13}C-n.m.r. spectroscopy, and partial acid hydrolysis showed that the polysaccharide had a β-(1→6)-D-galactan core branched on O-3 by side chains of α-(1→5)-linked L-arabinofuranosyl units, some of which were substituted in position O-5 by terminal D-galactopyranosyl residues. L-Rhamnose was found exclusively in terminal position as nonreducing unit.

THE COMPARATIVE EFFICACY OF YEAST POLYSACCHARIDES AS MACROPHAGE STIMULATORS

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The aim of this study - to compare effectiveness of several yeast polysaccharides Cryelan, Rhodexman (St.Petersburg, Russia) and chemically modified three fractions of β-1,3-carboxymethylglucan, CMG (Bratislava, Slovakia) as macrophage stimulators. In vitro according to NST-test all polysaccharides studied increased reduction of nitroblue tetrazolium by murine peritoneal macrophages (incubation at 37°C, 30 min, 150 μg/ml): CMG, 3rd fr. > CMG, 1st, CMG, 2nd fr. > Cryelan > Rhodexman. All drugs studied increased the phagocytic index in vivo (carbon clearance test), especially CMG fractions and Cryelan (25-50 mg/kg b.w. i.p. or i.v.). Kupffer cells play the main role in carbon particles phagocytosis in vivo, so it was concluded that stimulation of Kupffer cells occurred (1st-14th days). Electron microscopic study revealed increased number and quantitative density of Kupffer cells, increased relative volume of secondary and primary lysosomes of Kupffer cells (morphological symptoms of macrophage stimulation). Preliminary, 24 or 48 h before, injection of yeast polysaccharide (Cryelan, CMG) to mice or rats prevented depression of phagocytosis during cold exposure (-10 °C, 1 h, body temperature decrease 3°). It is possible to conclude that yeast polysaccharides are effective macrophage stimulators and can be used in pathology followed by macrophage depression. Immunostimulating action of yeast polysaccharides was noted only in cold stress (PFC/lien), but not in intact mice with administration of immunomodulator. So mechanism of protection included also IgM response, as was suggested.

STRUCTURAL CHARACTERIZATION OF YEAST GLUCOMANNAN AND ITS ANTIVIRAL ACTIVITY IN PLANTS

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Two glucomannans were isolated from the cell walls of *Candida utilis* and *Candida lambica*. In order to determine the detailed structure of glucomannans we subjected they to acetolysis that preferentially splits 1->6-α-linkages of the backbone and thus produces oligosaccharidic fragments. The structure of these fragments resulted of methylation analysis, ^{1}H and ^{13}C-NMR study. Therefore, it might be suggested that both glucomannans are highly branched, contain an 1->6-α-linked polymannosyl backbone with predominantly 1->2-α-linked side-chains. Some side-chains contain 1->3-α-glycosidic linkages. Glucose is situated as a terminal unit in side chains.

It was found that microbial polysaccharides have antiviral properties. It is supposed that cell wall components may induce phytoalexin synthesis as "endogenous elicitors". We studied the protective effect of these glucomannans to virus infection (tobacco mosaic virus and tobacco necrose virus) on cucumbers and beans. In comparison with other polysaccharides they were more effective than other tested ones (mannan, glucan).

RADICAL DEGRADATION OF HIGH MOLECULAR WEIGHT HYALURONAN: TESTING OF ANTIOXIDANT PROPERTIES OF HYDROPHILIC XENOBIOTICS

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A sample of high molecular weight hyaluronan (M_n= 3.17 x 10^5; or 4.95 x 10^5 Da) was exposed to the degradation effect of oxygen radicals generated chemicaly by two systems: (i) H_2O_2 + Fe^{2+}; (ii) ascorbic acid + Fe^{2+}. After a 3-h treatment the hyaluronan M_n value (3.17 x 10^5 Da) decreased in system (i) to 0.91 x 10^5 Da; in (ii) from 4.95 x 10^5 Da to 1.07 x 10^5 Da.

The kinetics of the hyaluronan depolymerization was followed to evaluate the antioxidant properties of hydrophilic xenobiotics: e.g. stobadin dihydrochloride was added into both systems in the amount of 1.65 x 10^{-6} mol. After 3 h the M_n value found in the system (i) was 2.4 x 10^5 Da; in (ii) was 2.6 x 10^5 Da.

An addition of the well defined antioxidant - stobadin dihydrochloride significantly reduced the degradation of high molecular weight hyaluronans. Thus it can be concluded that the two model systems (i) and (ii) are applicable for testing the antioxidant properties of hydrophilic xenobiotics.

CIRCULAR DICHROISM STUDY OF DERIVATIVES RELATED TO THE O-SPECIFIC POLYSACCHARIDE (O-SP) *VIBRIO CHOLERAE* 01.

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The LPSs of many Gram-negative pathogenic bacteria are both essential virulence factors and protective antigens. The O-specific PS of *Vibrio cholerae* 01 LPS is composed of approximately 15 repeats of α,(1-2)-linked *N*-(3-deoxy-L-*glycero*-tetronyl)-D-perosamine. Conformations of D-perosamine *N*-acylated with formic, acetic, 4-hydroxybutyric, 3-deoxy-L- or D-glycero-tetronyl acid in aqueous or in organic solvents were studied by circular dichroism (CD). The strong solvent dependence of the sign and intensity of CD spectra for amides bearing achiral acyl group is explained by solvent-induced changes in the orientation of the proximal hydroxyl group at C-3. Possibility of transition between conformers with a non-planar pyramidal arrangement of bonds at the amido nitrogen was also considered. The effect of solvent upon the CD spectra of chiral *N*-acyl substituents is less pronounced, the sign of CD did not change in different solvents. The CD spectra of O-SP isolated from serotypes Inaba and Ogawa were identical, similar to that of the monosaccharides. Negative sign of CD was observed in all solvents studied: water, acetonitrile+water (1:1), and trifluoroethanol+water (1:1). Thus, the solvent independent negative sign of CD appears to be characteristic of the L-*glycero* configuration of the *N*-acyl substituent. That the chirality of an *N*-acyl group affects the overall CD manifestation more strongly than does the solvent-induced conformation of the chiral arrangement may be useful to distinguish between these type of chiral compounds.

STUDIES ON ANTIGENIC VARIATION IN THE PHASE I LIPOPOLYSACCHARIDE OF COXIELLA BURNETII STRAINS

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Coxiella burnetii, the etiological agent of Q fever, is an obligate, intracellular parasite of eucaryotic cells. Biosynthesis of complete lipopolysaccharide (LPS) molecules, located on the surface of its outer membrane, is a host-controlled natural selection process for resistance to the microbicidal activities of the phagolysosome. Thus, chemical and antigenic diversity of the LPS molecules should vary according to the ability of the host to restrict growth of the microorganism.
Coxiella burnetii strains from a variety of clinical and geographical sources were propagated in chicken embryo yolk sacs. After purification, the C.burnetii cells were extracted with hot phenol-water procedure. The LPSs isolated were investigated by various methods of chemical analysis including colorimetric assays, GC-MS, and SDS-PAGE electrophoresis coupled with silver staining.
All strains possessed a phase I - type LPS being equivalent to smooth LPS. While SDS-PAGE profiles showed remarkable differences among the individual LPSs, the composition of their constituent sugars could not prove this trend. The latter results were supported also by the colorimetric assays for hexosamine, phosphate, and 3-deoxy-D-manno-2-octulosonic acid (Kdo) contents.

COMPARISON OF VARIOUS METHODS OF LIPOPOLYSACCHARIDE ISOLATION FROM COXIELLA BURNETII STRAIN PRISCILLA IN THE VIRULENT PHASE I

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In Coxiella burnetii, the causative agent of Q fever, a lipopolysaccharide (LPS) is located in the outer membrane of the bacterial cell. For structural studies, a highly purified and homogeneous LPS is needed, and therefore, application or development of a suitable isolation procedure is of great importance.
Four methods of isolation of the LPS from Coxiella burnetii strain Priscilla in virulent phase I have been examined. Each isolation method afforded a specific LPS portion of the polydisperse LPS system present in the outer membrane of C.burnetii cell. The hot phenol/water method was found the most efficient with respect to yield and chemical composition of the smooth (S) LPS that was shown to prevail in the outer membrane LPS macromolecules of C.burnetii.

CARBOXYMETHYLATED β-1,3-GLUCAN MODULATES ACETYLATED LOW DENSITY LIPOPROTEIN METABOLISM VIA INTERACTION WITH SCAVENGER RECEPTORS

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Effect of β-1,3-carboxymethylglucan (CMG, production of Chemical Institute, Slovak Academy of Sciences, Dr. J. Sandula) on metabolism of acetylated low density lipoproteins (Ac-LDL) known as ligand of scavenger receptors in peritoneal macrophage culture and on plasma clearance of Ac-LDL in rats was studied. It was found that CMG bloked the incorporation of [^{14}C]-oleate into cellular cholesterol esters in presence of non-labelled Ac-LDL (50 μg/ml) in cultured medium, the uptake and degradation [^{125}I]-AC-LDL, but not native [^{125}I]- LDL in a dose-dependent manner with complete inhibition at 10-20 nM. In contrast, other polysaccharides zymosan, endotoxin, nonmodified glucan and mannan Rhodexman had slight effect on Ac-LDL metabolism. [^{125}I]-Ac-LDL injected i. v. in rats was cleared from the plasma with a half-life of 3,4±0,2 min, while coinjection of [^{125}I]-Ac-LDL with CMG (10 mg/kg) i. v. significantly decreased the rate of Ac-LDL clearance. Two-fold preliminary injected CMG i. v. at a dose of 25 mg/kg 48 h and 24 h before determination increased the rate of [^{125}I]-Ac-LDL clearance by 60 per cent compared with control. It was concluded that CMG possessed high affinity to scavenger receptors in vivo and in vitro and preliminary injection of CMG can enhance the scavenger receptor function in vivo.

ALTERATIONS IN CELL WALLS AT LOW TEMPERATURE ACCLIMATION

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Alterations in cell wall polysaccharide composition of wheat seedlings during low temperature acclimation have been inverstigated. The growth braking observed as a result of temperature decrease (2^{o}) was accompanied by swift decline of ^{14}C-glucose incorporation into cellulose, hemicellulose and pectin fractions, but after 6-8 h the biosynthesis of matrix polysaccharides gradually recovered. After 20-24h of low temperature affect the intensity of biosynthesis of pectins reached the original level, biosynthesis of hemicelluloses exceeded that, while the cellulose biosynthesis still left at low level The most dramatic modifications were obtained in hemicellulose fractions. Analysis of monosaccharide composition and types of linkages of matrix polysaccharides showed the decrease of mixed glucan, xyloglucan and glucuronoarabinoxylan contents by the 6h of acclimation. The increase of 2-Ara and 3-Ara content usually reflect the rise of extencin content. The glycosidases connected with cell walls participate in processes of polysaccharide modifications. The temperature decrease resulted in short time activation of glucosidases, fucosidases and cellobiosidases extracted by NaCl, and glucosidases and fucosidases in EDTA extract of cell walls. Galactosidases activity in EDTA extract was decreased. Alterations observed in cell walls during low temperature acclimation first stage are similar to alterations obtained for other types of adaptation. The role of cell walls in low temperature acclimation of plants will be discussed.

SEPARATION OF OLIGOSACCHARINS OCCURRED NATURALLY IN PLANT TISSUES

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Until now there are some limits in the knowledge about oligosaccharin mode of action, whether they occur naturally and how they are generated in plant tissues at the right time, place and concentration. For such investigations it is an urgent need to find such right moment for attempt to separate oligosaccharins in their natural forms. Fast growing pea seedlings (8-9 days) and winter wheat seedlings placed for 6h at low temperature (2°C) have been used for separation of bioactive oligosaccharides. Cell walls were separated from the both plant homogenates and from the rest material neutral oligosaccharide fractions were separated using three steps of cation-exchange chromatography and gel-permeation chromatography. Two of eight pea fractions obtained inhibited the root development in buckwheat thin cell layer explants and showed anti- auxin properties in classical pea-stem elongation bioassay. These activities disappeared after treatment of fractions with glycosidases, what testify the glycan nature of activity observed. Monosaccharide analysis showed the presence of significant amount of xyloglucan fragments as well the presence of some arabinan/galactan, glucan and uronic fragments. Two wheat fractions among six obtained increased the hardiness of winter wheat seedlings till 20% determined by classical electrolyte leakage test. Data obtained support the hypothesis that oligosaccharins occur naturally.

PECTIC CELL WALL FRAGMENTS HAVE INFLUENCE ON BUCKWHEAT THIN CELL LAYER EXPLANTS RHIZOGENESIS

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Pectic fraction was separated from cell walls of Pisum sativum L. young seedlings (8-10 days). After acid hydrolysis and several steps of chromatographic separation a few active fractions were obtained. Three of them stimulated root development on thin cell layer explants by 3-6 times, at the same time some other fractions inhibited this process. All fractions tested showed their activities in concentrations ranging from 10^{-10}-10^{-8} M. Monosaccharide analysis showed that all active fractions had rather close sugar composition containing mainly arabinose and galactose and in minor amount-glucose and xylose. Fractions with inhibition activity contained uronic acids and rhamnose as well Each fraction contained 3-5 separate fragments with very close structure according to the results obtained by high-performance anion-exchange chromatography on a Dionex system. Using the endo-galactosidase and exo-arabinosidase we have got the evidence that the active fragments represented either galactan or arabinogalactan fragments.

Data obtained testify the existence bioactive arabinogalactan oligosaccharides like kwown already oligosaccharins, and support the assumption about existence of broad range of oligosaccharins with various structures and functions

Inhibition of auxin stimulated elongation of pea stem segments by galactoglucomannan-derived oligosaccharides

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Galactoglucomannan-derived oligosaccharides (GGMO) are recognized as inhibitors of auxin-induced long-term growth of pea and spruce stem segments. GGMO (d.p. 4-8) were found to inhibit the 2,4-D-induced short-term elongation of pea stem segments. Their inhibition effect was detected within 2h after start of incubation. Indirect dependence of GGMO inhibition effect and the concentration of 2,4-D has been observed.

Cytoplasmic arabinogalactan-protein complex of *Populus alba* L.

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Cytoplasmic arabinogalactan-protein complex isolated from poplar callus cultures influences the growth of pea stem segments stimulated by auxin, induces a variety of morphological responses in tobacco thin-cell-layers and Scorzonella leaf explants, and influences the regeneration of spruce protoplasts (Lišková et al. 1990).

Antiserum has been raised to the arabinogalactan-protein. This macromolecule has been characterized, and has a structure consisted of 1,3-linked-β-D-galactan backbone with side chaines of 1,6-linked-β-D-galactosyl residues and β-D-glucopyranosyluronic acid groups. The α-L-arabinofuranosyl residues were located mainly in the outer regions as nonreducing groups, as well as 1,5-linked inner chain residues and 1,3,5-linked branching residues. Small amounts of 1,4- and 1,4,6-linked galactose residues were also detected.

References

Lišková, D., Schinová, J., Kákošová, D., Kubačková, M., 1990: The regulatory effect of oligosaccharides in plant cells in vitro. VIIth Int. Congress on Plant Tissue and Cell Culture. Amsterdam, the Netherlands. p. 25

INDUCTION OF RESISTANCE AGAINST TOBACCO NECROSIS VIRUS BY XYLOGLUCAN OLIGOSACCHARIDES IN CUCUMBER COTYLEDONS

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Xyloglucans are an important class of cellulosic polysaccharides found in cell walls of all higher plants. They act as cell wall stabilizing molecules and may play a role in regulating plant cell growth. Their potential antiviral activity has not been described till now. We have investigated the activity of xyloglucan fragments in the induction of resistance against tobacco necrosis virus (TNV) in cucumber cotyledons (*Cucumis sativus* L. cv. Laura). Oligosaccharide fragments prepared from tamarind seed xyloglucan (TXG - contained a hepta-, octa- and nonasaccharide) and from pea xyloglucan (PXG - contained hepta-, nona- and decasaccharide) inhibited the development of necrotic local lesions induced by TNV on cucumber cotyledons. Inhibition of virus infection ranged from 44 % to 84 % when the xyloglucan fragments in amounts ranging from 10 µg to 100 µg per cotyledon were applied 24 h prior to virus inoculation. Oligosaccharides from both types of xyloglucan exhibited a similar efficiency. ELISA determinations of TNV proteins in cotyledons proved that XG - derived oligosaccharides applied 24 h prior to infection inhibited not only the disease symptoms but also the virus multiplication in parallel. Our results corroborate with the assumption of the role of endogenous elicitor derived from the plant cell wall in the mechanisms of plant resistance to viruses.

EXTRACELLULAR GLYCOPROTEIN FROM THE YEAST CRYPTOCOCCUS LAURENTII.

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Differential preparation of culture medium *Cryptococcus laurentii var. laurentii* with Fehling's solution [1] was used for isolation of extracellular glycoprotein. Fehling's precipitable fraction β (high mannose glycoprotein) is characterized by molar ratios of mannose : glucose : xylose : ribose 1 : 0.13 : 0.10 : 0.02 : 0.07. The fraction β contained 5.2% of protein. 43% of threonine and 30% of serine residues of this fraction was glycosylated by O-linked oligosaccharides. The released O-glycosidic oligosaccharides were chromatographed on HPLC. Mannooligosaccharides with α-linked galactose on terminal non-reducing ends were found. 95% of saccharide part of fraction β is N-linked on protein part of glycoprotein.

SACCHARIDE ACCEPTORS OF THE GALACTOSYLTRANSFERASES FROM CRYPTOCOCCUS LAURENTII.

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Expression of membrane-associated and cytosolic galactosyl transferase (GalTase) activities in the yeast *Cryptococcus laurentii* depends on the phase of growth. Both galactosyl transferases differ not only in cellular localization and expression, but also in enzymatic properties. Suitable saccharide acceptors of membrane-associated and cytosolic GalTases were examined in the enzyme reaction with UDP-galactose as a galactosyl donor. Cytosolic form of GalTase is inhibited by N-Acetyl-D-Glucosamine and lactose whereas for membrane-bound GalTase the both sugars are the best acceptors. The steric effect of methyl group in α- or β-position of various derivated saccharides used as the acceptors in UDP-Gal transferase reaction has not been observed. These findings suggest that the expression of two different galactosyl transferase could play a regulatory role in glycosylation mechanisms.

POTENTIAL OF GLUCOAMYLASE IN THE SYNTHESIS OF OLIGOSACCHARIDE ANALOGUES

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Glucoamylase (EC 3.2.1.3) is known to catalyze condensation of D-glucose into various α-linked oligosaccharides. We have investigated the substrate requirements in such reactions with *Aspergillus niger* glucoamylase using eight different monodeoxy- and monodeoxy-monofluoro analogues of D-glucose. At 4M initial monosaccharide, the highest conversion into oligosaccharides was obtained with 2-deoxy-D-glucose (~17 %) and 3-deoxy-D-glucose (~8 %) . The yields of oligosaccharides increased by about 5 % in the presence of diethylene glycol diethyl ether (V. Laroute and R. M. Willemot, Biotechnol. Lett. 11, 249, 1989). Enhanced yields appears to result from ether-mediated removal of water, which, in turn, served to concentrate the reactants in the aqueous phase. Dependent on the amount of ether used, the aqueous phase became syrupy, and phase discrimination was difficult to discern. An analogous observation has been reported recently in glucoamylase-catalyzed condensations of D-glucose (L. Cantarella et al., Enzyme Microb. Technol. 16, 383, 1994). Several D-glucose analogues that did not afford any products on incubation with glucoamylase (e.g. , 2-deoxy-2-fluoro-D-glucose and 3-deoxy-3-fluoro-D-glucose) served as glycosyl acceptors in reaction mixtures containing D-glucose. The yields of mixed-sugar oligosaccharides formed in mixtures containing 2M monosaccharides were in the range 1 to 6 %. The oligosaccharides formed from 2-deoxy-D-glucose were isolated and subjected to structural analysis.

ROLE OF YEAST EXTRACELLULAR GLYCOPROTEINS IN THE CRYOPROTECTION AND OSMOTOLERANCE OF YEASTS

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Yeasts are able to produce glycoprotein components into grown medium. The quantity and quality of these substances are dependent on the cultivation conditions. Glycoproteins tested by us were composed of the largest heteropolysaccharide tail and less protein tail.
The viability of cells during thawing depends on the composition of cryoprotective medium as well as combination of penetrating and nonpenetrating cryoadditives in which cells suspension was frozen. The concentration of glycoprotein 0.27 % (w/v) was sufficient for successful storage of yeasts in liquid nitrogen.
During batch growth of strains of genera *Dipodascus* and *Dipodascopsis* was shown that the cells tolerance to osmotic dehydration (influenced by NaCl stress) is dependent also on the production of glycoproteins.
Yeast extracellular glycoproteins have a function of osmotic - buffer in both storage and grown mediums. The protective effect of these glycoproteins could be explained by their electrostatic, hydrophilic, and hydrophobic interactions with water and outermost layer of cells.

THE INFLUENCE OF GLYCOSYL AMINE OF D-GALACTOPYRANURONIC ACID ON THE ACTIVITY OF EXOPOLYGALACTURONASE FROM CARROTS

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Exopolygalacturonase [E.C. 3.2.1.67] purified from carrots was inhibited by glycosyl amine of D - galacto-pyranuronic acid. K_m and V of the action of this enzyme on pectic acid and penta-(D-galactosiduronic acid) and K_i and V_i for various concentrations of glycosyl amine in such system were established. The results showed mixed type of inhibition. This can be partially explained by inhibition of exopolygalacturonase by non-substituted D-galactopyranuronic acid, which is a predominant product of this enzyme action. This makes app. 50% of the glycosyl amine inhibition.
The effect of this inhibitors on the exopolygalacturon-ase activity using substrates with various degree of polymerization [di-, tri-, tetra-, penta-(D-galactosid-uronic acid) and pectic acid] was investigated to get an information connecting the active site of this enzyme.

STRUCTURE OF HYDROLYSIS OF GLYCOSIDIC LINKAGE BY TWO ENDO-β-1,4-XYLANASES FROM *TRICHODERMA REESEI*

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Methyl β-glycoside of β-1,4-xylotriose (a generous gift of Dr. P. Kovac) [1], further as $MeXyl_3$, was used as a non-reducing substrate to investigate the stereochemistry of hydrolysis of β-1,4-xylopyranosidic linkage by purified endo-β-1,4-xylanases (EC 3.2.1.8) of *Trichoderma reesei*, employing ^{1}H-NMR-spectroscopy. The fungus produces one acidic species (pI 4.8-5.5), designated as EXI, and one alkaline species (pI 8.5-9.0), designated as EXII. Both enzymes, purified to homogeneity [2], were found to cleave $MeXyl_3$ predominantly to MeXyl and xylobiose (Xyl_2) as the main reducing product. The stereochemistry course of the cleavage was followed by ^{1}H-NMR-spectroscopy of the products formed in D_2O using thoroughly deuterized enzymes and the substrate. The enzymes were used at a concentration ensuring rapid cleavage of the substrate so that the configuration of the newly formed reducing end could be established earlier than the anomeric equilibrium due to mutarotation. Plotting of the intensity of signals of the α- and β-anomeric protons of the reducing end of Xyl_2 versus time clearly showed that both *T. reesei* EXs liberate β-anomer of xylobiose, i.e. a product with anomeric configuration identical with that of the cleaved glycosidic linkage. This means that both enzymes belong to the so called, retaining glycanases that use a double displacement reaction mechanism of hydrolysis of glycosidic linkage [3].

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A COLORIMETRIC METHOD FOR THE ASSAY OF XYLOGLUCAN-ENDOTRANSGLYCOSYLASE ACTIVITY

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The enzyme xyloglucan-endotransglycosylase (XET) degrades xyloglucan (XG) molecules predominantly by a transglycosylation mechanism, i.e. by endo-splitting the β-(1-4)-linked polyglucose backbone of XG molecule and transferring the newly formed reducing end to the nonreducing end of another XG molecule or of XG-derived oligosaccharide (1,2). The transglycosylation mechanism implies that practically no net formation of reducing ends takes place in the course of reaction, which precludes the use of reductometric colorimetric methods to determine the XET activity. Currently used methods for estimation of the XET activity are based on viscometry (3), determining of the changes in the distribution of M_r's of the substrate XG or the formation of transglycosylation products to fluorescent pyridilamino oligomers of XG by HPLC (4). Another method employs the measurement of radioactivity incorporated into XG when radioactive ^{3}H-labelled XG-oligosaccharides are used as glycosyl acceptors (1). All these methods suffer from disadvantage by being time-consuming, expensive, or both.

Our method for the assessment of XET activity is based on the ability of XG-derived oligosaccharides (XGO's) to promote the XET-catalysed degradation of XG by serving as additional glycosyl acceptors (2). The assay is performed with polymeric XG as the substrate and added XGO's used to promote the transglycosylation reaction. In the course of reaction, the XG is degraded to molecular species with M_r < 10 kDa and looses its ability to form coloured complex with iodine (6). This method is about 10 [illegible] more sensitive than viscometry, is rapid, inexpensive, and has the advantage that la[illegible] samples can be processed simultaneously.

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MICROASSAY FOR KINETIC PARAMETERS DETERMINATION OF XYLANASES.

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For the determination of reducing-sugar, the Nelson-Somogyi (1) and the copper-bicinchroninate (2) assays have been adapted for microplate uses, here we report the adaptation of the assay of Lever (3) based on *p*-hydroxy-benzoic acid hydrazide.

Kinetic parameters were determined using 1.0 ml total volume of a xylan solution allowing multiple time point samples (100 μl) from each tube. The volume of the assay could be easily decreased down to 400 μl and still allowing multiple time point samples which minimize the consumption of valuable substrate such as xylooligosaccharides.

- Using 0.25 % reagent, the assay show linearity between 1-100 nmoles with xylose as standard.
- For kinetic determination using xylooligosaccharide which caused high background of reducing-sugar, the assay could be adapted by increasing the concentration of reagent. This gave a more sensitive determination of liberated reducing-sugar over the background and moreover, the linearity of the assay is conserved and not affected upon dilution of the reaction mixture after development using up to 5% of *p*-hydroxy-benzoic acid hydrazide.
- The assay is less dependent to the chain-length dependence of xylooligosaccharide than the traditional dinitrosalicylic acid.

Application of the microassay will be presented using wild type and mutant xylanases A of *Streptomyces lividans*.

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PRODUCTION OF EXTRACELLULAR β-MANNANASES FROM YEASTS AND YEAST-LIKE MICROORGANISMS

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530 yeasts and yeast-like microrganisms of 73 different genera were screened for the production of extracellular endo-β-1,4-mannanase. Microorganisms were cultivated after stabbing on a solid agar medium containing covalently dyed galactomannan (Ostazin Brilliant Red-galactomannan) as the only carbon source. Decolourizing of the substrate around the cell colonies as a result of substrate depolymerisation was observed with 22 strains of 3 genera: *Aureobasidium*, *Pichia* and *Stephanoascus*. β-Mannanase-positive strains were further grown in a liquid medium containing 1% locust bean gum as a carbon source, and β-mannanase activity in the cell-free culture fluid was determined after 3 and 6 days. The best producers of the enzyme were found to be strains of *Aureobasidium pullulans*.

SOYBEAN SEED PECTINESTERASE

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Methanol accumulates in axis tissues of maturing soybean seeds and correlates with preharvest seed deterioration (1). Accumulation of methanol appears to be associated with the enzymic demethylation of pectin methyl esters by pectinesterase (EC 3.1.1.11). In order to characterize pectinesterase in soybean seeds, the activity of this enzyme in axis and cotyledon tissues was followed in several stages of seed development. The highest pectinesterase (PE) activities in both axes and cotyledons were observed between 45 and 60 DAF (days after flowering). PE activities per g fresh weight in axes were 20-25 times higher than in cotyledons. A PE bound to cell-wall fragments was characterized in soybean cultured cells (2), but no information was reported for soluble PE, extractable from homogenized tissue with water or 0.5 M sucrose. Up to 40% of the total PE activity present in cotyledons and axes of soybean seeds was in the "soluble" form. Axes from soybean seeds at 45 to 60 DAF were used for purification and characterization of soluble and cell-wall bound PE (extractable with 1 M NaCl from homogenized axis residues after extraction with water, 0.5 M sucrose, 1 M sucrose and water). Purification was achieved by homogenization and extraction (as mentioned above), concentration by ultrafiltration, precipitation with ammonium sulfate (30-80% saturation), dialysis, gel filtration on Sephadex G-75 columns, and ion exchange chromatography on CM Sepharose CL-6B. The purification of both soluble and bound PE was followed by isoelectric focusing (IEF) on ultrathin layers of polyacrylamide gel with simultaneous detection of protein and PE activity. It was possible to follow seven bands exhibiting PE activity with pI between 6.0-9.5 in extracts with 1 M NaCl of total homogenate. Differences in the IEF patterns of bound and soluble PE were observed. Whereas bound enzyme exhibited the more basic PE bands (pI 8-9.5) the soluble enzyme had the more active bands at pI 6.5, 7.0 and 7.5. The M_r of soluble and bound PE was the same - close to 33,000. The pH optimum was 7.8 for both enzyme fractions.

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MULTIPLE FORMS OF *Aspergillus species* POLYGALACTURONASE. GLYCOPROTEINS?

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The primary structure of PG III. form of *Aspergillus* sp. polygalacturonase shows potential N-glycosilation site (Asn246 - Val - Thr248). This sequence is strictly conserved with corresponding sequences of other known primary structures of fungal polygalacturonases. The results of other indirect determinations indicate the presence of small N - linked glycans, too.
The interaction of polygalacturonase forms with Concanavalin A bound onto cellulose was investigated. The binding and elution conditions were optimalized. The affinity of all forms of polygalacturonase present in used commercial preparation to Concanavalin A seems to be the same. This indicate, at least, the similarity of the glycosilation of individual forms, however the amino acid sequenced are different.
The possibility to use bound Concanavalin A for bio-affinity chromatography of polygalacturonase and for oriented immobilization of this enzyme is discussed.

CHANGES IN DISTRIBUTION OF SHORT PECTIC POLYSACCHARIDES INDUCED BY ALKALINE IONS IN THE NITELLA CELL WALL

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Treatments of isolated cell walls of Nitella flexilis , a freshwater alga whose walls are qualitatively like dicotyledonous cell walls, by monovalent ion solutions induce a large pectin solubilization(1). We used monoclonal antibodies (2F4) to monitor the changes in the wall immunogold distribution of the pectin polysaccharides at the electron microscopic level, before and after the walls in the Ca^{2+} form were exchanged with NaCl and LiCl solutions. Our antibodies are specific for a conformational epitope of homogalacturonic acid induced by calcium ions (2). In the whole cell wall equilibrated with 5mN $CaCl_2$ the labelling pattern enabled two pectinaceous domains to be observed. The wall outer layer is characterized by a larger density of labelling than the inner part of the wall. After hydrolysis in situ of pectin of the thin sections by 50 mM NaOH, one observed a net increase of labelling in the outer part of the wall confirming that the recognized epitope is a short segment of polygalacturonic acid. In contrast, in the walls equilibrated with 100 mM NaCl or LiCl, the thin sections were almost devoid of any gold particles. Only a little labelling is still observed in the outer part of the NaCl treated walls. These data corroborate the biochemical analyses of the extracted polymers in the monovalent ion solutions that show the presence of polymers largely composed of rhamnogalacturonan whose average degree of polymerization does not exceed 25. These results also show that in the primary part of the Nitella cell wall, there is a large fraction of relatively small polyuronides that may be solubilized by alteration of the wall ionic phase.

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MATHEMATICAL DESCRIPTION OF CONVERSION OF THE SIMPLEST SACCHARIDES

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The numerical mathematical method for solution of a complex reaction system completely describing conversions of the simplest saccharides-trioses is presented. It is the system of two reversible and simultaneously of two irreversible first-order reactions which is solved by the least-squares method, iterations, and their mutual combination.

The solution is based on the fact, that the time dependence of concentrations can be expressed by characteristic equations. The roots of these equations, i.e. m_1 and m_2, were found by iteration as the minimum of the function $R(m_1,m_2)$. The final solution of the system is achieved by finding the minimum of the sum of the squares of concentration deviations, calculated by the least-square method.

The approximative method of solution is conditioned by achieving reacting equilibrium and equidistance course of time dependence of concentrations relatively for a long time [1]. The advantage of the presented numerical method in comparison with the above mentioned one is in the possibility to analyse also slowly reacting systems, without obtaining the equilibrium.

This numerical method has been successfully applied to kinetic analysis of transformations of trioses not only in alkaline, but also for in weak acid media[2].

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Molecular-biological and chemical approaches to the study of glycosidic moieties of major royal jelly glycoproteins.

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The properties of individual glycoproteins of major royal jelly protein fraction (MRJPF) as well as the composition of their glycosidic moieties were studied by the combined molecular-biological and chemical approaches:

1. The cDNA clones were isolated from the expression cDNA library of 8 days old nurse honey bee heads by immunoscreening with antibodies raised against MRJPF (1). The amino acid sequences of individual proteins related to MRJPF were deduced from nucleotide sequences of these cDNAs. The consensus sequences for glycosylations sites were predicted by computer analysis. Though the studied glycoproteins have 57% of protein sequence identity they markedly differ in the number of the potential glycosylation sites.

2. The monosaccharide composition of oligosaccharides linked to threonin and serin and polysaccharides linked to asparagin was analysed by gas chromatography.

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Author's Index

About the Castle...

The first documents about the existence of Smolenice Castle date back to early 15th century. The Castle served as a watch-fortress to protect the Carpathian pass at Trstín of the so-called *Via bohemica*, a historical route linking the regions of south-eastern Europe with the countries in the West. In the period of 16th to 18th centuries, the Castle was the flowering seat of aristocratic Erdödy family. By the end of the 18th century the Castle fell into decay and during Napoleonic wars, it was destroyed by fire. For the whole century later, the Castle remained as an unhabitable ruin.

The present Castle of Smolenice was built in pseudogotic style as a residence by the count Josef Pálffy (1853-1920), in the years 1911-1914. The last owner, Jozef Pálffy, Jr. spent his life predominantly abroad, leaving the administration of his properties to foreign personell whose husbandry resulted in loss. Increased debts and great sums of unpaid taxes have in 1943 prompted the state to put the domain and the industrial plants (sawmills and a chemical plant) of the Pálffy family in the Smolenice area under compulsory state administration and ownership. Much of the Castle interiors were lost or destroyed during the 2nd World War.

In 1963, the Castle was completed and renovated by the state and allotted to the Slovak Academy of Sciences to become the House of Scientific workers; it serves as the meeting place for conferences, symposia and for recreation. The Castle is surrounded by beautiful natural park framed by the hills of the Small Carpathian Mountains.